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TITLE OF THE INVENTION

Molecular Analysis Probes, Systems and Methods, including DNA Sequencing

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Respectfully submitted,

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Molecular Analysis Probes, Systems and Methods, including DNA Sequencing

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TECHNICAL FIELD

[01] The present invention relates to methods and apparatuses for analyzing molecules, particularly polymers, and molecular complexes with extended conformations. In particular, the methods and apparatuses are used to identify sequence information in molecules or molecular ensembles, which is subsequently used to determine structural information about the molecules.

BACKGROUND ART

[02] . The development of high-throughput DNA sequencers in the 90's have helped launched the genomic revolution of the 21st century. Almost on a monthly basis, one research group or another is announcing the complete sequencing of a biologically important organism. This has allowed researchers to cross reference species, finding shared and/or similar genes, and allowing the knowledge of molecular biologists in all the various fields to come together in a meaningful way.

[03] However, current techniques in DNA sequencing are far too tedious, tying up the valuable time of researchers. Even the fastest, most advanced DNA sequencers can at most process a few hundred thousand base pairs a day. The Human Genome Project took over 10 years to complete, indicating that current DNA sequencing technology still has a long way to go before it can be used as a diagnostic tool. Considering that there are about 3 billion DNA base pairs in the mammalian genome, and current sequencing technology is capable of sequencing about 2 million DNA base pairs per day, it would still take over 4 years to sequence the human genome.

[04] Known nucleic acid sequencing methods are generally based on chemical reactions that yield multiple length DNA strands cleaved at specific bases. Alternatively, other known nucleic acid sequencing methods are based on enzymatic reactions that yield multiple length DNA strands terminated at specific bases. In either of these methods, the resulting DNA strands of differing length are then separated from each other and identified in strand length order. The chemical or enzymatic reactions, and the methods for separating and identifying the different length strands, usually involve repetitive procedures. Thus, there remains significant limitations on the speed of DNA sequencing using conventional technology.

[05] Despite these limitations, an incredible collaborative heroic effort was undertaken for the Human Genome Project. It took many years and billions of dollars to obtain the sequence to the human genome. It would be highly desirable to provide a method and system that reduces the time and effort required would represent a highly significant advance in biotechnology. Indeed, frontier advances are required to increase the efficiency and speed of DNA sequencing if we are to expand the genome databases that presently exist to include a genome library including flora and fauna. Certain flowering plants have 100 times more base pairs than the human genome, so existing sequencing technology must be leaped for a new frontier of sequencing systems.

Pores

[06] One particular type of sequencing method relies on passing strands of DNA through pores. For example, U.S. Patent Nos. 5,795,782, 6,015,714, 6,267,872, 6,362,002 6,428,959 6,465,193 6,617,113, 6,627,067, 6,673,615, 6,746,594 6,870,361 describe various sequencing techniques and apparatus based on pores and flow of DNA fragments through pores. In general the prior art pores have thickness that cannot

directly resolve with high spatial resolution without some other indirect deconvolution of the date resulting from changes in ionic conductivities. It further cannot be used for large DNA fragments. Further, it is very time consuming. In general, for an ultra fast DNA sequencing system, there are many limitations with pore based systems.

[07] Therefore, it would be desirable to provide an improved system and method of analyzing extended objects such as linear polymers (including proteins, DNA and other biopolymers).

BRIEF SUMMARY OF THE INVENTION

[08] Using the herein nano-nozzles, a DNA sequencing method is presented that may sequence the entire Human Genome in a matter of minutes. Realizing and optimizing this technology opens new vistas for human endeavors, and enables practical applications that are nearly limitless. Culturing bacteria would be a thing of the past. Whenever faced with an unknown organism, not only could its exact species be determined immediately, but also its entire genotype, including new mutations or signs of genetic engineering. This process is based on utilization of the nanoscale probes, e.g., in the form of electrodes, nozzles, funnels, or other suitable probes. These nanoscale probes are coupled with detection of ultra small and ultra fast signals. This sets the course ofr the development of the ultimate sensor, not only for DNA, and RNA, but also to sequence denatured proteins (amino acid sequence of polypeptides).

[09] As discussed above, current DNA sequencing technology is most often based on electrophoresis and polymer chain reaction (PCR). PCR is used to create varying lengths of the DNA in question, which is then subjected to electrophoresis to resolve the size

differences between the DNA fragments. However, this technique faces several bottlenecks. First, although PCR is useful in amplifying the amount of DNA material, it is time consuming, requires numerous reagents, including the use of an appropriate primer. Second, electrophoresis speed is dependent on the applied voltage. But the applied voltage cannot be further increased unless heat dissipation is similarly increased. Also, electrophoresis gel is only capable of resolving a small dynamic range (<500bp). This requires splitting an organism's genome apart for sequencing and then re-assembling the pieces.

[10] Instead of relying on electrophoresis to resolve the DNA sequence, the proposed sequencing technology is based on nano-electronics.

[11] The herein system and method relies on probes having resolution capabilities less than the dimensions of the objects to be analyzed. Further, systems and methods are provided herein that allow for accurate measurement of the portions of the specimens to be analyzed, such as individual monomers in a polymer chain.

DETAILED DESCRIPTION OF THE FIGURES

General

[12] Described herein is a novel system and method for analyzing extended object specimens. The system includes analytical probes configured and dimensioned such that the edge of the probe has a thickness direction that is spatially smaller than the desired resolution. Further, in one embodiment, the analytical probe has a width dimension that is much larger than the thickness of the extended object. In other embodiments, the analytical probe has a path in the width direction that is much larger than the thickness of the extended object.

Definitions

Extended Object

[13] In the preferred embodiment, the "extended object" to be analyzed is a polymer. A polymer, as used herein, is a compound having a linear backbone of individual units linked together by covalent bonds. Preferably, the backbone is unbranched. The term "backbone" is given its usual meaning in the field of polymer chemistry. The polymers may be heterogeneous in backbone composition, thereby containing any possible combination of individual monomer units linked together, e.g., peptide- nucleic acids (PNA), having a polypeptide-like backbone, based on the monomer 2-aminoethyleneglycin carrying any of the four nucleobases: A, T, G, or C. In a preferred embodiment, the polymers are homogeneous in backbone composition and are, e.g., nucleic acids or polypeptides. A nucleic acid as used herein is a biopolymer comprised of nucleotides, such as deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA). A protein or polypeptide as used herein is a biopolymer comprised of amino acids. In the most preferred embodiment, the extended object is a single stranded (denatured) DNA molecule with a rigid structure.

[14] It will be appreciated by one skilled in the art that the system described herein for monomer level resolution may be used for other molecular level detection, e.g., for single small molecules, single monomers, oligomers, or other nano-scale structures.

Probe

[15] Further, as used herein, the term "probe" refers generally to any device used to interact with individual monomers or groups of molecules of the extended object. Probes may be formed of various configurations and materials to be described further herein.

Detectable Interaction

[16] Further, as used herein, the term "detectable interaction" refers generally to an interaction between the probe and a portion of the extended object. The portion of the extended object with which a detectable interaction occurs may include individual monomers or groups of monomers.

General System

[17] Referring now to Figure 1, a schematic overview of the system of the present invention for analyzing extended object specimens is shown. The system 100 generally includes a specimen platform 128, a probe set 130 and a detector sub-system 132. The platform 128 is operably coupled to a motion controller 138, for controlling motion of the platform. Alternatively, the specimen may be moved within the platform. In a further alternative, the probe set (and optionally the associated detector sub-system) may be moved relative to the platform with the specimen. Further, the system 100 includes a bias sub-system 136 for control of field application (voltage applied across base and probe). When the hybridization event occurs, there is an increase in current.

[18] In certain embodiments, a low detection voltage may be applied in a constant manner across the probe set and the platform. However, biased voltage application is preferred to minimize or eliminate noise.

[19] Data regarding the specimens is collected and processed by a processor sub-system 134, which is coupled to an output sub-system (e.g., a display, data port, etc.) 140.

[20] In operation, a specimen such as a single stranded polymer (e.g., a denatured strand of DNA) is directed through a channel in the platform. The probe set detects characteristic features of the polymer specimen, preferably detecting characteristic about each sequential monomer in the specimen polymer. The specimen is moved relative the

probe set in a controlled manner, e.g., by step motion to allow the probe set to obtain characteristic information about each monomer or group of monomers. The sequence information is collected, processed and outputted.

Probe Configurations

[21] The probes used herein may be formed of various materials and configurations. For example, probes may be in the form of wells or nozzles having a tip for dispensing materials to facilitate analysis of the specimen. The nozzles configured for dispensing materials may include conductive inner walls, or a conductive element disposed within a material holding region, in order to facilitate measurement and other voltage applications across the probe. In other examples, the dispensing materials are within a conductive medium to facilitate measurement and other voltage applications across the probe.

Knife Edge Probe

[22] Referring now to Figure 2, a probe 202 is depicted. Probe 202 is particularly well suited for analyzing extended object specimens such as biopolymers. Probe 202 is characterized by a tip 204 thickness *t*, a tip 204 width *w*, and a height (not identified in the Figure). Importantly, the tip thickness *t* is dimensioned to obtain the desired resolution of the system. For example, when information regarding individual monomers of a DNA strand is desired, the thickness *t* should be less than the nucleotide spacing on the strand (about 0.5 nm). Still further, probe 202 has a width dimension *w* that is preferably much greater than the width of the specimen. This ensure that as the probe passes over the specimen, landing error associated with typical probe analysis systems is eliminated. As shown, in certain preferred embodiments, the probe has a shape that provides a larger end 206 opposite the tip 204. This can, for example, reduce electrical resistance of the probe when end 206 serves as a contact region. Further, the larger end

206 serves to facilitate introduction and dispensation of materials from the probe when the probe is in the form of a nozzle filled with suitable material, as described further herein.

Scanning Probe

[23] Referring now to Figure 3, a probe 302 is depicted. Probe 302 is particularly well suited for analyzing extended object specimens such as biopolymers. Probe 302 is characterized by a tip thickness *t*, a tip width *w*, and a height (not identified in the Figure). Further, probe 302 is positioned within a suitable sub-system 308 to impart motion to the probe generally in the direction of the width *w* along a path *pw*. Similar to probe 202, the tip thickness *t* is dimensioned to obtain the desired resolution of the system. The width dimension *w* of probe 302 is not critical. However, the path width *pw* is preferably much greater than the width of the specimen. This ensure that as the probe passes over the specimen, landing error associated with typical probe analysis systems is eliminated.

Probe Shape

[24] Note that although the shape of the probe is depicted in the shape of a prism with ends in the form of an isosceles triangle, the probe may also be in the shape of a prism with ends in the form of a right triangle (Figure 4A), a trapezoid)(Figure 4B), or a rectangle (Figure 4C, e.g., the shape of the probe is a parallelepiped). Further, the probe may be in any other irregular shape, for example, with curved or rounded walls, as shown in Figures 4D and 4E. In certain preferred embodiments, as described above, the tip thickness *t* is dimensioned to obtain the desired resolution of the system.

Probe Set

[25] Figures 5A and B show an enlarged isometric view and side view, respectively, of a probe set 530 including probes 542, 544, 546, and 548, and a specimen polymer strand

550 upon a platform 528. In certain preferred embodiments, polymer strand 550 is a biopolymer such as a nucleic acid (e.g., DNA). Figure 5C shows an enlarged sectional view through any one of probes 542, 544, 546, or 548. Figure 5D shows a top view of the base platform 528, showing an exemplary channel 552. As shown in Figures 5C and 5D, in certain embodiments, a *measuring* voltage is applied across each probe 542, 544, 546, 548, and platform 528, denoted by reference numerals 554a and 554b, respectively. As the polymer strand 550 passes under an activated probe (e.g., a probe with a measuring voltage applied thereto), detectable interactions occur as described in further detail herein.

[26] Figures 6A-6D show a probe set 630 formed according to embodiments of the present invention. The probe set includes, e.g., a 1 x 4 array (although it is understood that this may be scaled to any size n x m nozzles) of probes 642, 644, 646, 648.

[27] In certain embodiments, these probes 642, 644, 646, 648 are in the form of nozzles, e.g., having tips 654 associated with wells 656, as shown in Figure 6B and 6C. Generally, the wells having widths in the y direction greater than the widths of the nozzle tips. Figure 6D shows a sectional view of the nozzle array.

[28] The probe set 630 may be embedded in a body 658. The material for the probes or nozzles, and the body, may be the same or different materials, and may include materials including, but not limited to, plastic (e.g., polycarbonate), metal, semiconductor, insulator, monocrystalline, amorphous, noncrystalline, biological (e.g., nucleic acids or polypeptides based materials or films) or a combination comprising at least one of the foregoing types of materials. For example, specific types of materials include silicon (e.g., monocrystalline, polycrystalline, noncrystalline, polysilicon, and

derivatives such as Si3N4, SiC, SiO2), GaAs, InP, CdSe, CdTe, SiGe, GaAsP, GaN, SiC, GaAlAs, InAs, AlGaSb, InGaAs, ZnS, AlN, TiN, other group IIIA-VA materials, group IIB materials, group VIA materials, sapphire, quartz (crystal or glass), diamond, silica and/or silicate based material, or any combination comprising at least one of the foregoing materials. Of course, processing of other types of materials may benefit from the process described herein to provide probes and bodies of desired composition.

Extended Opening Channel

[29] In another embodiment, and referring now to Figures 7A-C, the probes according to the present invention may be configured about more than one portion of the specimen to be analyzed, for example, in the form of an extended opening channel which interrogates from more than one side of the specimen.

[30] Presently, it is known to coax DNA fragments through a pore for the purpose of measuring a change in ionic conductivity. The pore is often part of a system of ionic fluids, whereby ionic conductivity change is measured across regions of ionic fluids separated by a membrane and/or layer having one or more pores. For example, as described in the background of the invention, patents 6,870,361, 5,795,782, 6,267,872, 6,362,002, 6,627,067 describe such pores.

[31] However, according to the extended opening channel system 700 of the present invention, a specimen 750 is passed through an extended opening channel 701. Each extended channel opening includes several probes formed according to any one or more of the various embodiments herein. By using an extended opening channel which interrogates from more than one side of the specimen, accuracy may be enhanced, and signal is increased.

[32] As discussed below with respect to Figures 8A-8B, these extended opening channels may be configured in arrays in a 2 dimensional or 3 dimension configuration, which presently known pore based sequencing systems cannot achieve.

Probe Arrays

[33] Referring now to Figure 8A, a serial probe array 877 is shown. The probe array includes Q serial probe sets 830. In general, extended objects to be analyzed may be passed through the Q serial probe sets 830. The Q serial probe sets may be homogeneous or heterogeneous.

[34] For example, using homogeneous probe sets 830, each probe set may include various individual probes optimized for adenine, cytosine, guanine, and thymine.

[35] Further, the array of probe sets may be heterogeneous, whereby, for example, one probe set is optimized for adenine, a second optimized for cytosine, a third for guanine and a fourth for thymine.

[36] These serial arrays would not be possible using conventional known techniques, for example, based on pores as described in the background of the invention. Importantly, redundancy is readily achievable in a serial configuration of the present invention, whether the system is formed of serial heterogeneous probe sets, serial homogeneous probe sets, or combinations thereof.

[37] Referring now to Figure 8B, a parallel and serial probe array 878 is shown. The probe array includes M x N channels of Q serial probe sets 830. This probe array 878 may be very useful for high speed parallel processing of extended objects to be analyzed. The probe sets 830 within the array 878 may be homogeneous or heterogeneous. The extended objects may be the same or different. In general, extended objects to be analyzed may be passed through the Q serial probe sets. An M x N array of extended

objects, which may be the same or different, are passed through the M x N arrays of Q serial probe sets 830.

Probe type for various Detectable Interactions

[38] The above described probes may be used in various configurations. Certain probes may be in the form of wells with dispending tips. Certain probes may be in the form of nano-nozzles. Certain probes may be in the form of nano-funnels. Certain probes may be in the form of electrodes for measuring detectable interactions. Certain probes may be in the form of materials that result in detectable interactions such as a system of correlating biological materials that create hybridization events with the extended object to be analyzed.

Nucleotide Filled Well

[39] In certain embodiments, and referring now to Figures 9A and 9B, the basic principle is described, wherein a DNA chain (or other protein or extended object to be analyzed) 950 upon a base 928 is passed underneath four nano-sized nozzles 942, 944, 946 and 948 (or arrays of nozzles, e.g., as shown in Figure 9B). The four funnels or nozzles 942, 944, 946 and 948 are filled with adenine, cytosine, guanine, and thymine molecules respectively. Due to the complementary structures of adenine and thymine, and of guanine and cytosine, a hybridization event between nucleotides on the DNA chain and the nucleotides in the nozzle will occur when the correct pairs come into contact. This hybridization results in a lower energy state and charge transfer, which can be detected via an ammeter. This is because the conductivity between the nozzles and the electrode ground plate will be affected, thereby altering the current between the nozzle and the ground plate. Figure 9B shows an exemplary array setup, e.g., that may average out noise and increase SNR. These features will help in assuring an excellent SNR.

[40] Note that the above described probes may also be formed with one or more conductors therein for increase signal detection capabilities. For example, the conductor may be layered within or upon an inner wall of the probe or nozzle well and tip/

Solid State nucleotide

[41] Referring to Figure 10, an embodiment of a system 1000 having probes formed of solid state nucleotide materials is shown. A probe set 1030 is depicted wherein each probe 1042, 1044, 1046, 1048 is formed of a solid state nucleotide, e.g., adenine, cytosine, guanine, and thymine molecules respectively. A solid state nucleotide may be manufactured on thin films, and formed as probes using the various manufacturing methods described herein or other thin film manufacturing techniques. Preferably, these SSN have a single molecule thickness at the probe tip, so that a desirable monomer scale resolution is maintained. These films may be formed in the nozzle wells, e.g., by layering during the manufacturing process prior to slicing. In preferred embodiments of a DNA sequencing system herein, the nozzles are formed with a tip dimension of less than about 0.5 nanometers to resolve corresponding monomers.

[42] It is known that DNA strands may be condensed on substrates. In the herein probes, single species nucleotide strands may be condensed in the form of lines of films. Referring to Figure 10B, these may be formed on a substrate (M), such as a conductive substrate, Referring to Figure 10C, condensed single species nucleotide strands may be sandwiched between substrates (M).

[43] The films resulting from Figures 10B or 10C may be used directly as the probes. Alternatively, these films may be slices and attached to metallic "knife blades". In a further alternatively, they may be folded, whereby exposed condensed single species nucleotides serve as the probe.

Metal conductor

[44] Referring now to Figure 11, a system 1100 is shown using a metal conductors as probes 1131. The probe may be formed of a suitable conductor material. Further, probes in the form of nozzles may be filled or layered with metal conductor material. The metal may be platinum, gold, or other suitable metal or non metal conductor. In preferred embodiments of a DNA sequencing system herein, the conductor probes formed to a tip dimension of less than about 0.5 nanometers to resolve corresponding monomers.

[45] In one method of using a probe 1131, stimuli (e.g., a voltage) is applied across the subject nucleotide within the subject strand, and a characteristic I vs. V curve may be obtained. For example, Figure 12 shows an exemplary representation of characteristic curves for various monomers adenine, cytosine, guanine, and thymine (A, C, G and T).

[46] In certain embodiments, a single probe 1131 may be used as described in Figure 11. In other embodiments, a probe set may be used, whereby bias waveforms across different electrodes may be varied to adjust sensitivity for expected specimen portions or monomers. For example, a four-probe probe set may be used for identifying A,C,T,G components of biopolymers such as DNA strands. Further, identical waveforms may be applied whereby multiple probes are used for redundancy. These may be gated or un-gated, depending on the application.

Metal plus known nucleotide stand, preferably same species

[47] Referring now to Figure 13A, an embodiment of a system 1300 having probes formed of conductor with a known material strand attached to the edge of the probe, particularly the "knife edge" probe, e.g., described above with respect to Figures 2 and 3. For example, a probe set 1330 is depicted wherein each probe 1342, 1344, 1346, 1348

has a known nucleotide strand, e.g., adenine strand, cytosine strand, guanine strand, and thymine strand respectively.

[48] In a preferred embodiment, a single strand/single species nucleotide strand is provided. It is stretched and attached to the tip of a conductor probe.

[49] The known nucleotide strand may be attached to the tip if the conductor probe by various nano- or micro- manipulation means.

[50] In one embodiment, magnetically attractive molecules, referred to as "magnetic beads", may be attached at opposing ends of the known strand to facilitate manipulation. A nano-manipulator magnet system may be used to stretch the strands for attachment to the probe set. For example, this is shown with respect to Figure 13B. Further, this configuration ensure that as the probe passes over the specimen, landing error associated with typical probe analysis systems is eliminated.

[51] With a single-strand, single-species chain attached at the probe tip, when the tip encounters a specimen portion or monomer that is capable of forming a hybrid pair with the probe species, bond energies associated with the hybridization event enhances the resonance activity being measuring.

Open Well or Funnel

[52] Referring to Figure 14, an embodiment of a system 1400 having probes formed as open wells or funnels is shown. A probe set 1430 is depicted wherein each probe 1442, 1444, 1446, 1448 is formed as an open well or funnel. This open well or funnel may be used as a path for various probe activities, for example, generated by sources 1482, 1484, 1486, 1488.

Particle beam

[53] Particle beams, as used herein, can be made directly into nano probes or indirectly through the funnel described herein. They include ion beams, electron beams and photon beams such as x-ray, ultraviolet, IR, visible. In the event that the excitation photon beams have wavelengths large than the probe diameter, the use of evanescent fields that extend only to the width scale of the beam (probe) will be utilized.

Electron beam emission

[54] In another embodiment, an electron beam emitter is focused and shaped to provide a nano-scale resolution beam. They can be tuned in energy. This tunability can give one selectivity in directly interacting with the specimen to be analyzed. Electron beams may be used as the probe for the systems of the present invention.

[55] It is known in the electron optics art that atomic scale resolution may be achieved with SEM, TEM, and STEM since the beams themselves can be made nano-scale as the probing beams. In preferred embodiments of a DNA sequencing system herein, the electron beams are focused to a sectional dimension of less than about 0.5 nanometers to resolve corresponding monomers. The electron beam may be a line beam (analogous the probe of Figure 2), or electron beam scanning may be employed (analogous the probe of Figure 3, although it is to be understood that the funnel need not be moved, only the beam).

[56] Referring to Figure 14, the electron beam may be inserted through the funnel. This minimized the need for nano-scale resolution electron optics required for direct electron beam formation at the atomic scale.

[57] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of electron beams, electric fields appropriately placed may cause these beams to bend toward the funnel tip. Alternately, secondary electron emission may be created from inner funnel wall surfaces which lead to the creation of a beam that exits the funnel tip.

Ion beam

[58] In another embodiment, a focused ion beam emitter with nano-scale resolution known in the art may be used as the probe to interact with the specimen. They can be tuned in energy. This tunability can give one selectivity in directly interacting with the specimen to be analyzed. Further, the ion beams may be based on H+, He+, Ge+, Ga+, or other suitable ions of substances that may be formed into beams that have specific selective interaction with the specimen to be resolved.

[59] Referring to Figure 14, the ion beam may be inserted through the funnel. This minimized the need for nano-scale resolution electron optics required for direct electron beam formation at the atomic scale.

[60] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of electron beams, electric fields appropriately placed may cause these beams to bend toward the funnel tip. Alternately, secondary electron emission may be created from inner funnel wall surfaces which lead to the creation of a beam that exits the funnel tip.

X-rays

[61] X-ray beams, such as an x-ray laser beam, may be used as the probe for the systems of the present invention. In preferred embodiments of a DNA sequencing system herein, the x-ray beams are focused to a sectional dimension of less than about 0.5 nanometers to resolve corresponding monomers. For example, the electron beam system described above may be used to generate nano-scale x-ray beams in a manner known in the art.

[62] Further, referring to Figure 14, an x-ray beam (directly or indirectly) may be inserted through the funnel. This minimized the need for nano-scale resolution x-ray and electron optics required for direct electron beam formation at the atomic scale.

[63] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of x-ray, the inner surfaces of the funnel may be made of multi-surface to achieve interference reflection, or may be of single crystal using Bragg reflection properties, or may be grazing incidence angle rejection until the rays reach the funnel end.

[64] To avoid stray x-rays that may interfere with excitation and/or measureant and increase noise, the inner and outers surfaces of the funnel as appropriate may be coated with x-ray absorbers.

Probe on Flexible Membrane

[65] In another embodiment, and referring now to Figures 15A and 15B, a bendable membrane material having a nano-scale probe attached thereto is provided. The nano-scale probe may one of the aforementioned probes such as a known nucleotide strand or other molecular probe. Preferably the bendable membrane material include a metallic

surface with the probe attached thereto to facilitate current measurement. Using a suitable MEMS or other plunger, the metal membrane is pulsed to make contact with the specimen to resolve it.

[66] As with the other probe types described herein, a 2D or 3D array may be provided. Further, these arrays may include homogeneous or heterogeneous probe types.

Stimuli

[67] In a preferred embodiment, the probes in the form of electrode conductors and/or other stimuli are applied in a gated manner. This reduces the signal to noise ratio thereby allowing for increased sensitivity and ability to resolve the sequence of the specimen.

Gated application of stimuli

[68] In a preferred embodiment, the probes in the form of electrode conductors and/or other stimuli are applied in a gated manner. This reduces the signal to noise ratio thereby allowing for increased sensitivity and ability to resolve the sequence of the specimen.

[69] Detection of a hybridization event may be accomplished in certain embodiments by observing variations in resonant capacitance. For example, an AC bias is imposed through a probe and a grounded platform (or alternatively AC bias may be imposed through the platform and the probes are sequentially grounded). The AC bias will alternately deplete and accumulate the specimen. The change in capacitance ΔC is recorded, for example, using a lock-in technique. The measured value ΔC may be the value across the entire C-V curve when larger AC voltages are used, or measured value ΔC may be the differential capacitance dC/dV when smaller AC bias voltage is used. The variation in the load across the specimen occurs due to characteristics of the portion of the specimen to be resolved such as a monomer on a polymer strand, or due to creation

of a hybridization event when the probe includes a hybrid pair counterpart. This load variation changes the resonant frequency of the system.

[70] Electrical conductors as probes according to preferred embodiments of the present invention, formed as described above with respect to Figures 2 and 3 above (e.g., in the configuration with a very fine tip compared to the back end, or a "knife edge") also serves to lower the resistance of the conductor.

[71] Various embodiments of stimuli application are possible. 1) voltage only; 2) voltage plus light (AND gate) (light is a noise reduction means); 3) synchronization with gating, pulsed voltage, light, and current gate leads to substantial noise reduction; 3a. controlled stepping; 3b) apply voltage and light (AND gate) – light of different wavelengths to enhance inelastic tunneling current; 3c) apply current gate (measure with ammeter); 4) kT (thermal energy) may be reduced under low temperature operating conditions, e.g., T between 4 and 100 K.

[72] Gated detection serves to minimize noise and allow for precise resolution of the extended object. Gated detection is necessary to ensure the detection of picoamp level currents in the presence of noise. One effective strategy is to apply all of the stimuli in the proper sequence, in the form of pulses. The pulse widths and heights are adjusted to achieve optimum results. The levels of voltage will be in the 10s of millivolts up to about 1 volt. The pulse durations may be about 1 nanosecond to about 1000 nanoseconds, or longer if necessary.

[73] The protocol for gated detection is described in the following steps: 1) apply a pulse to step the specimen relative to the platform to a position to measure a portion of or a nucleotide of the specimen; 2) subsequent application of an electric field to provide

contact between the specimen and the probe; 3) optional application of a laser pulse; 4) application of tunneling device voltage pulse; 5) applying a pulse to open the switch to the current measure device; 6) repeating 1-5 to measure the subsequent portion of the specimen or nucleotide to sequence. These steps 1-5 are synchronized pulses synchronized to a master clock. In the event that particle beams are applied, or intensifiers, these will also have appropriately applied excitation pulses to activate them synchronized with said clock. These gated synchronized methods allow one to measure the detectable interaction with a high signal to noise ratio.

Detection

[74] Detection of the portion of the specimen under examination may occur by various contribution. In general, the detection schemes allow for molecular level (or detection of one or more monomers, or certain groups of monomers, in an extended object to be analyzed) identification of monomers within a chain.

[75] In a single strand specimen analysis systems having probes that induce a hybridization event, detection contribution includes elastic tunneling, inelastic tunneling, resonantly enhanced tunneling, and/or capacitance.

[76] The elastic tunneling contribution in systems having probes that induce a hybridization event is generally due to the tunneling interaction variations that occur due to the distance between hybridized species. When a hybridization event occurs, the distance between the hybridized monomers (nucleotides) is modulated as the bond is created. As the tunneling barrier thickness decreases, tunneling probability increases and thereby increases the tunneling contribution. This will be manifested in the increase of conductance as measured in the current-voltage characteristics of the hybrid bond. When no hybridization event occurs, the distance between the probe capable of inducing a

hybridization event and the specimen nucleotide remains relatively large, and hence the elastic tunneling contribution is relatively low.

[77] The inelastic tunneling contribution in systems having probes that induce a hybridization event is based on increased bond energies, especially hydrogen bond energies. During a hybridization event, as electrons tunnel, the electrons lose energy by exciting the hydrogen bond created as a result of the hybridization event. This leads to a tunneling contribution at a voltage correlating to the energy of the bond. When no hybridization event occurs, there is no hydrogen bond created, therefore there is no inelastic tunneling to excite such a bond, and therefore no conductance contribution should be observed.

[78] The resonantly enhanced tunneling contribution in systems having probes that induce a hybridization event is based on measurement of excited bond energies, particularly hydrogen bonds. Stimuli such as light application is applied. A resonantly enhanced tunneling contribution may be observed when a light source such as a laser having a suitably tuned wavelength excites the hydrogen bond created upon hybridization. Hydrogen bonds from the hybridization events can be excited by tuning a laser beam to the same energy as the bond. This will enhance the detection of both the elastic and inelastic tunneling contribution and add a resonant enhanced tunneling contribution to the measurement current. Further, noise is minimized with suitable gating as described herein since the pulsed application of the laser light source is synchronized with application of a voltage and during the opening of the measurement current sensor. These simultaneous interactions have the effect of a logical "AND" gate.

[79] The capacitance contribution in systems having probes that induce a hybridization event is based on enhanced permittivity. Since the tunneling area is very small, the application of a laser beam tuned at or near the bond energy creates a resonantly enhanced permittivity at the hybridized pair. This in effect is like a quantum capacitance. This quantum capacitance, added to a specific inductive element, an RF resonant circuit, or a RF resonant cavity, results when the hybridization even occurs. For example, the inductive element, RF resonant circuit or RF cavity are excited and can give a very large signal. Since RF frequencies are at higher frequencies than the DC voltages, there is low noise in that region (avoiding the 1/F noise).

[80] In a single strand specimen analysis systems having probes that do not induce a hybridization event, detection contribution includes inelastic tunneling, resonantly enhanced tunneling, and/or capacitance.

[81] Detection based on the elastic tunneling contribution is not particularly effective without a probe that induces a hybridization event. Since the distance between the probe (in a system that does not induce a hybridization event) and the specimen nucleotide reaming relatively large, the elastic tunneling contribution is relatively low for all nucleotides. Therefore, an elastic tunneling contribution is not suitable for measurement detection system when using probes that do not induce hybridization events.

[82] However, detection of measurement current variances due on inelastic tunneling contribution may be used. Since there is no hybridization event (e.g., the probes are formed of conductors or other style that does not induce a hybridization event), we rely on the inherent resonance of each nucleotide to be analyzed.

[83] Further, the resonantly enhanced tunneling contribution is suitable, wherein a light source (e.g., laser wavelength) is tuned to the inherent unique resonances of the nucleotides to be analyzed. The nucleotides to be analyzed are be excited by tuning a laser beam to that unique resonance, which will enhance the detection of the inelastic tunneling contribution and other contributions to the current measurement. Further, noise is minimized with suitable gating as described herein since the pulsed application of the laser light source is synchronized with application of a voltage and during the opening of the measurement current sensor. These simultaneous interactions have the effect of a logical "AND" gate.

[84] The capacitance contribution in systems having probes that do not induce a hybridization event is also based on enhanced permittivity analysis. Since the tunneling area is very small, the application of a laser beam tuned at or near the inherent unique resonance energies creates a resonantly enhanced permittivity of the signature. This in effect is like a quantum capacitance. This quantum capacitance, added to a specific inductive element, an RF resonant circuit, or a RF resonant cavity, results when the signature energy occurs. For example, the inductive element, RF resonant circuit or RF cavity are excited and can give a very large signal. Since RF frequencies are at higher frequencies than the DC voltages, there is low noise in that region (avoiding the 1/F noise).

Strategy for Error Reduction

[85] As descried herein, array of probes sets in 2d or 3d arrays can measure and re-measure the same sample., This is possible due to the low cost techniques. Further, multiple channels for parallel systems may be used.

[86] Gated electronic techniques are also used herein with a pulse protocol that is applied to ensure minimize noise.

[87] Many sensing techniques for determining a hybridization event include elastic quantum mechanical tunneling; inelastic quantum mechanical tunneling; resonantly enhanced tunneling; resonantly enhanced quantum capacitance in a tank circuit to boost the signal of hybridization events; fast cooling techniques to reduce noise (for example, such as the system that utilized liquid He or liquid N_2 droplet cooling); ionic conductivity; quantum mechanical tunneling electron emission; photon emission, which can be amplified by photon multiplier techniques. Any one or more of these techniques may be used in conjunction with the herein described high spatial resolution (e.g., nucleotide monomer level resolution) probes, probe sets or probe arrays as a novel direct sequencing system.

[88] Another aspect of the present invention to minimize error is the extended configuration (e.g., "knife edge") as described above with respect to Figures 2 and 3.

[89] In systems herein where metal contacts or probes are used to measure currents and voltages from small structures such as the monomers of the specimen, four probe tunneling devices as are known in the art (e.g., shown in Figure 17, are preferred to minimize contact and lead resistance. Also, preferred probe configuration provide for a larger end opposite the tip, for example, as shown with respect to Figure 2. Further, all contacts the probe are preferably much larger than the tip. This can, for example, reduce electrical resistance of the probe when end serves as a contact region.

[90] Optimum specimen resolution and speed may be achieved by optimizing the detection system to increase the measurable signal, namely, ensuring that enough

electrons are involved, and minimizing the ambient noise. The tunneling current densities involved, in such small tunneling areas (e.g., .5 square nanometers), makes it possible to involve 10s of electrons and 10s of picoamps. This is achieved by allowing the time aperture to excite and detect each nucleotide in the order of 1 – 1000 nano-seconds. This can achieve the desired result of sequencing the whole Human Genome of 3×10^9 base pair in a time of about 1 second to a few minutes.

[91] We have allowed for even higher speed and fewer electrons to be involved whereby intensification/amplification sub-systems are used to intensify few electrons or photons into a measurable signal.

[92] Gated detection further serves to minimize the errors in the system. This is necessary to ensure the detection of picoamp level currents in the presence of noise. One effective strategy is to apply all of the stimuli in the proper sequence, in the form of pulses. The pulse widths and heights are adjusted to achieve optimum results. The levels of voltage will be in the 10s of millivolts up to about 1 volt. The pulse durations may be about 1 nanosecond to about 1000 nanoseconds, or longer if necessary.

[93] The protocol for gated detection to minimize noise is described in the following steps: 1) apply a pulse to step the specimen relative to the platform to a position to measure a portion of or a nucleotide of the specimen; 2) subsequent application of an electric field to provide contact between the specimen and the probe; 3) optional application of a laser pulse; 4) application of tunneling device voltage pulse; 5) applying a pulse to open the switch to the current measure device; 6) repeating 1-5 to measure the subsequent portion of the specimen or nucleotide to sequence. These steps 1-5 are synchronized pulses synchronized to a master clock. In the event that particle beams are

applied, or intensifiers, these will also have appropriately applied excitation pulses to activate them synchronized with said clock. These gated synchronized methods allow one to measure the detectable interaction with a high signal to noise ratio.

[94] One important factor of this method is obtaining a sufficient signal to noise ratio. The system is preferably gated and synchronized such that the ammeter will only detect a signal when a nucleotide is directly below a nozzle. The bias applied may be positive, negative, or even alternating, as to maximize the change in conductivity. Cooling may be desirable to reduce the thermal noise. Alternatively, each DNA or protein strand may be passed under several arrays of nozzles, thereby averaging out the noise. Certain embodiments show array configurations, e.g., that may average out noise and increase SNR. These features will help in assuring an excellent SNR.

[95] However, if we assume a 10 picoamp current change under one applied volt, and 10 nanoseconds for detection, the signal is orders of magnitude larger than the thermal noise, even at room temperature. The sequencing speed would be enormous. Allowing 30 nanoseconds to move a nozzle from one nucleotide to the next (a speed of about 1 cm/sec), it would take only 40 nanoseconds to sequence one base pair, which is equivalent to 1.5 Billion base pairs a minute.

[96] The above described DNA sequencing is enabled by creating a probe having tip dimensions on the order of about 5 Angstroms, for example, utilizing the above referenced and described nozzle manufacturing methods.

Methods of making the probes

[97] There are various methods of making the probes, probe sets and probe arrays described herein. Co-pending U.S. Non-provisional Application Serial No. 10/775,999 filed on February 10, 2004 (and corresponding PCT Application PCT/US04/03770)

entitled "Micro-Nozzle, Nano Nozzle and Manufacturing Methods Therefor", incorporated herein by reference, describe various techniques for manufacturing probes in the form of nozzles or funnels are described. These techniques may be modified to provide other probe configurations and probe types described herein.

MFT

[98] Various probes and configurations thereof may be manufactured with the use of Applicant's multi-layered manufacturing methods, as described in U.S. Non-provisional Application Serial Nos. 09/950,909, filed September 12, 2001 entitled "Thin films and Production Methods Thereof"; 10/222,439, filed August 15, 2002 entitled "Mems And Method Of Manufacturing Mems"; 10/017,186 filed December 7, 2001 entitled "Device And Method For Handling Fragile Objects, And Manufacturing Method Thereof"; PCT Application Serial No. PCT/US03/37304 filed November 20, 2003 and entitled "Three Dimensional Device Assembly and Production Methods Thereof"; U.S. Patent No. 6,857,671 granted on April 5, 2005 entitled "Method of Fabricating Vertical Integrated Circuits"; U.S. Non-provisional Application Serial Nos. 10/717,220 filed on November 19, 2003 entitled "Method of Fabricating Muti Layer Mems and Microfluidic Devices"; 10/719,666 filed on November 20, 2003 entitled "Method and System for Increasing Yield of Vertically Integrated Devices"; 10/719,663 filed on November 20, 2003 entitled "Method of Fabricating Muti Layer Devices on Buried Oxide Layer Substrates"; all of which are incorporated by reference herein. However, other types of semiconductor and/or thin film processing may be employed.

[99] Referring to Figure 18, an enlarged cross section of stacked layers 110 used to form the micro and nano nozzles having wells and tip portions as described herein, cut to desired tip length, is shown. The layers 138 have been processed to form the wells 130

and nozzle tip regions generally by deposition of a layer 138 of material capable of being selectively removed (e.g., etched) therein (the well) and thereon (the shelf at the top of the well), as described herein. The materials capable of being selectively removed for the plateau and and/or the well may be the same or different. The wells and plateaus have various dimensions that will characterize the nozzle array ultimately formed. The nozzle has a tip length NL, a tip opening height NO, and a period P.

Nanolithography

[100] Various probes and configurations thereof may be manufactured with the use of Applicant's microlithography and nanolithography tools and methods, as described in U.S. Non-provisional Application Serial No. 11/077,542 filed on March 10, 2005 entitled "Nanolithography and Microlithography Devices and Method of Manufacturing Such Devices".

Signal to Noise Ratio

Base – Specimen Configuration

[101] In certain embodiments, the specimen may be within a channel of the base. A channel may include suitable fluid, or the specimen may be coaxed through a channel with little or no fluid.

[102] In other embodiments, the specimens may be embedded within the base, e.g., in a biochip.

Electron Intensifier

[103] In certain embodiments, an electron or photon intensifier such as a micro-channel intensifier may be used. For example, referring to Figure 16A and 16B, these embodiments are shown.

[104] Referring to Figure 16A, the probe emitter interacts selectively with the specimen in an elastic or inelastic manner, whereby energy is lost, and the event lead to the release

of photons or electrons that have specific energy indicative of the nature of the molecule or monomer. These electrons or photons may be too few to be measured directly. Therefore, the invention herein provides for an intensification or amplification sub-system such as micro-channel plate intensifiers known in the art, e.g., night vision goggles or photo-multipliers.

[105] Referring now to Figure 16B, where the probe is either metallic and/or a molecular probe, interaction with the specimen may be through inelastic tunneling current. Rather than measuring this tunneling current directly, it is possible to provide a sub-system for allowing either photons or electrons to be emitted. The photons or electrons to be emitted may occur upon a hybridization event, or by applying suitable voltage energy to emit inelastic electrons indicative of the spectra of the specimen. This electron is also detected my an intensifier/amplification sub-system described above with respect to Fig. 16A.

[106] Referring now to Figure 16C, an array of intensifiers/amplifier sub-systems as described with respect to Figure 16A or 16B may be provided. For example, the exciting probe beams or other probe types may be tuned or optimized from a particular monomer, for example, in a DNA sequencing system, A, T, C, G, such that the electrons or photons are emitted are signatures of each type of nucleotide to be detected.

Systems

[107] Referring now to Figure 18, an embodiment of an ultra-fast DNA sequencing system 700 is shown. The sequencing system uses a nozzle array 710, as described herein. Further, the sequencing system uses a nano-metrology system 720 to precisely guide denatured DNA strands across the individual nozzles in the nozzle array.

[108] Referring now to Figure 19, a schematic of major components of the ultra-fast DNA sequencing system 700 are shown. A nano-nozzle set array platform 730 upon an N-channel specimen array platform 728 is operably connected to a detector array 732 associated with a processor 734, generally for determining instances of hybridization events induced by the biases applied via a gated bias array control 736. The DNA specimens are maintained and displaced in relation to the array with a stepped motion control 738, which is also operably connected to the processor 734. The array platform 728 is movable at a velocity of about 0.1 to about 1 cm/s. Preferably, as shown, the motion is in a stepped manner, as described herein. The sequencing results are shown on a sequence display 740.

[109] The stepped motion is important in preferred embodiments, as the motion and number of steps helps maintain knowledge of position on the ssDNA, and ultimately the position of hybridization events. The stepped motion may be from about 5% to about 100% of the nozzle opening dimension, preferably about 10% to about 25% of the nozzle opening dimension.

[110] The gating is also important in preferred embodiments, as extremely synchronized current measurements, bias, motion steps, or other excitations are crucial to ultra-fast real time DNA sequencing.

[111] Referring now to Figure 21, a top view of the ultra-fast DNA sequencing system 700 is shown. The DNA specimens are denatured and maintained within channels 744.

[112] Referring now to Figures 22A-B (wherein Figure 22A is a section along line A-A of Figure 21), each channel 744 includes biasing systems for applying voltages across the DNA samples. As described in more detail herein, hybridization events induce

measurable current variations across each of the nanonozzles within the nanonozzle set array platform. Preferably, the alignment between the nanonozzles and the channels is extremely precise.

[113] Referring now to Figure 23, detailed section views of the sequencing process are shown. The nanonozzle set array platform includes nanonozzles with wells, or nucleotide reservoirs, of A,C,T and G molecules. The strands are moved along the channel and molecules from the nucleotide reservoirs interact with the molecules of the strand through the nozzle. These molecules hybridize with one other molecule (e.g., A with T, C with G) as is known in the art.

[114] Referring now to Figure 24, detailed views of hybridization events are shown. Only a hybridization event at the nanonozzle results in a measurable current pulse.

[115] Referring now to Figure 25, it is shown that, of all possible 16 combinations of A,T,G and C, only four produce current pulses upon a hybridization event.

[116] As mentioned above, only a hybridization event produces a measurable (nanoseconds) current pulse at the nozzle. For proper operation, the following principles apply.

•All excitation sources, detectors and stepped motion are synchronized.

•Synchronized steps should be a fraction of the nozzle opening size (e.g., on the order of 5 nanometers).

•Nozzle locations should be known with nanometer or sub-nanometer precision in relation to a known reference position.

•Nanometer alignment is very important to optimal operation.

•Vibrations and other agitations should be minimized.

•A sub-system is provided to measure very low amplitude nanosecond pulses.

•For continuous real time measurement of millions, or even hundreds of millions, of base pairs, a wide dynamic range sub-nanometer stepper is preferred.

•To calibrate the system, it is desirable to use known samples.

[117] Referring now to Figure 26, a reference position and precision nanometer metrology system is shown. A reference position probe (RPP), e.g., formed of platinum or other suitable material, or in the form of a nano-light guide, or other excitation means, is included in the nanonozzle array set. The positions of each nanonozzle relative the RPP is shown. This probe provides a spatial zero when sequencing commences.

[118] Referring now to Figure 27, the stepped motion of ssDNA is shown relative to a known position of the RPP.

[119] To assist the denaturing in conjunction with the precise stepwise motion, the DNA strand can be straightened bay various methods. In one embodiment, electrostatic fields may be used to attract the negatively charged strands. In another embodiment, a magnetically attractive bead may be applied to an end of the DNA strand, and the strand pulled with magnetic force. In a further embodiment, viscosity optimization may be employed, such that while dragging the strand through a liquid proximate or in the channel, it will straighten upon optimal dragging velocity and fluid viscosity conditions. Further, hydrophilicity may be used, e.g., by suitable material treatment at or in the nozzles and channel walls, to attract nucleotides. In other embodiment, hydrophobicity may be used, e.g., by suitable material at or in the nozzles and channel walls, to maintain the fluid within the channel.

[120] Thus, as shown and described, the herein system including nano-nozzles and nano-nozzle arrays are very well suited for ultra fast real time DNA sequencing operations.

[121] While preferred embodiments have been shown and described, various modifications and substitutions may be made thereto without departing from the spirit and scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustrations and not limitation.

CLAIMS

What is claimed is:

1. A probe for analyzing an extended object, the extended object having plural sub-objects, the probe comprising a body having an edge, the edge having a thickness less than a relevant dimension of one of said sub-objects, and a length substantially greater than a relevant dimension of one of said sub-objects.

2. A probe as in claim 1 wherein said probe includes a material that hybridizes with at least one known sub-object of said plural sub-objects.

3. A probe for analyzing an object, the probe comprising a body having an analyzing region, the analyzing region having a dimension less than a relevant dimension of one (or more) of said objects, and a width substantially greater than a relevant dimension of one of said objects.

4. A probe for analyzing an object, the probe selected from group consisting of nozzle filled with liquid, an particle beam, electron beam, x-ray beam, a light beam, or a metal, the probe including an analyzing region, the analyzing region having a dimension less than a relevant dimension of one (or more) of said objects, and a width or a path width substantially greater than a relevant dimension of one of said objects.

5. A probe for analyzing an object comprising a source of a probe beam, the probe beam having an analyzing dimension less than a relevant dimension of one (or more) of said objects, and a width or a path width substantially greater than a relevant dimension of one of said objects

6. A detection system comprising

   a. a probe as in any of claims 1-5

   b. a base for supporting an extended object

   c. a sub-system for applying a stimuli across the probe and the base wherein a detectable interaction occurs upon passage of an extended object between said probe and said base.

7. A device for analyzing an extended object comprising an

   one or more probes as in any of claims 1-5;

   wherein the probe imparts excitation on a portion of the extended object to be analyzed.

8. The device as in claim 7, wherein the portion of the extended object to be analyzed is a monomer within a polymer chain.

9. The device as in claim 7, wherein the excitation comprises an electric field.

10. The device as in claim 7, wherein the excitation comprises an electric field induced hybridization event between the portion of the extended object and a portion of the probe.

11. The device as in claim 7, wherein the excitation comprises an electric field and a light source.

12. A device comprising an

    one or more probes as in any of claims 1-5;

    a handling sub-system for handling an extended object

    a stepping sub-system for stepping the relative position of the extended object and the array of probes;

a current measurement system for ascertaining a measurable current pulse upon existing of a hybridization event between one or more of said probes and one or more of the sub-objects within the extended object.

13. A device comprising an

one or more probes as in any of claims 1-5;

a current measurement system for ascertaining a measurable current pulse upon existence of a hybridization event between one or more of said probes and one or more of the sub-objects within the extended object.

14. The device as in claim 12 or 13, wherein said measurable current pulse includes contributions based on elastic tunneling, inelastic tunneling, resonantly enhanced tunneling, capacitance, or any combination of the foregoing contributions.

15. A device comprising an

one or more probes as in any of claims 1-5;

a current measurement system for ascertaining a measurable current pulse between one or more of said probes and one or more of the sub-objects within the extended object.

16. A device comprising an

one or more probes as in any of claims 1-5;

a handling sub-system for handling an extended object

a stepping sub-system for stepping the relative position of the extended object and the array of probes;

a current measurement system for ascertaining a measurable current pulse

upon existing of a hybridization event between one or more of said probes and one or more of the sub-objects within the extended object.

17. The device as in claim 15 or 16, wherein said measurable current pulse includes contributions based on inelastic tunneling, resonantly enhanced tunneling, capacitance, or any combination of the foregoing contributions.

18. The device as in any one of claims 12-17, wherein stimuli application and detection measurement are synchronously applied.

19. The device as in claim 18, wherein a pulse is applied to step the specimen to a position to measure a portion of the specimen; an electric field is applied to provide contact between the specimen and the probe, application of a tunneling device pulse; and application of a pulse to open a switch to the current measure device.

20. The device as in claim 19, wherein a light source is synchronously applied prior to opening the switch to the current measuring device.

21. The device as in any one of claims 18-20, further comprising an electron or photon intensifier.

22. A method of analyzing a specimen comprising

providing a specimen to be analyzed relative a probe according to any one of claims 1-5;

applying a pulse to step the specimen to a position to measure a portion of the specimen;

applying an electric field to provide contact between the specimen and the probe;

applying a tunneling device pulse; and

applying of a pulse to open a switch to the current measure device.

23. The method as in claim 22, further comprising applying a light source prior to opening the switch to the current measuring device.

24. An array of probes as in any of claims 1-5.

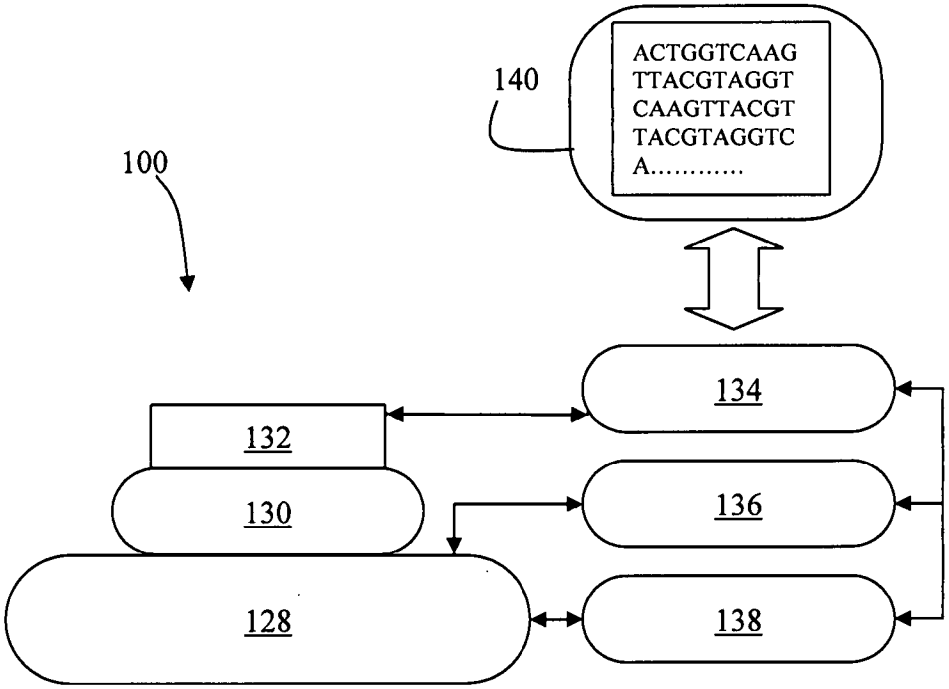
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FIGURE 1

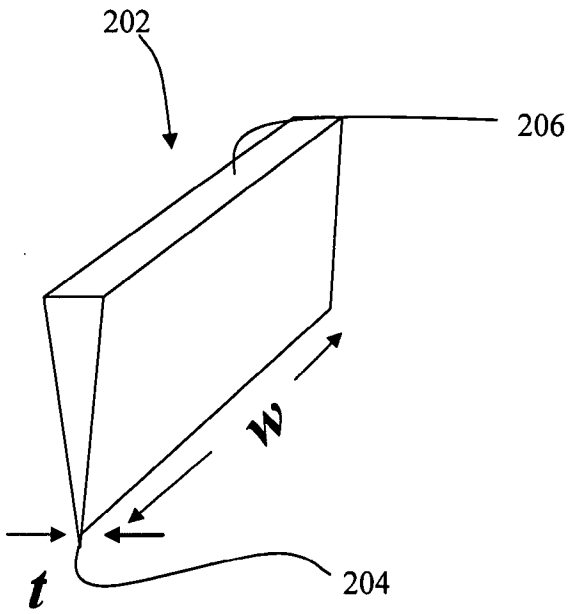


FIGURE 2

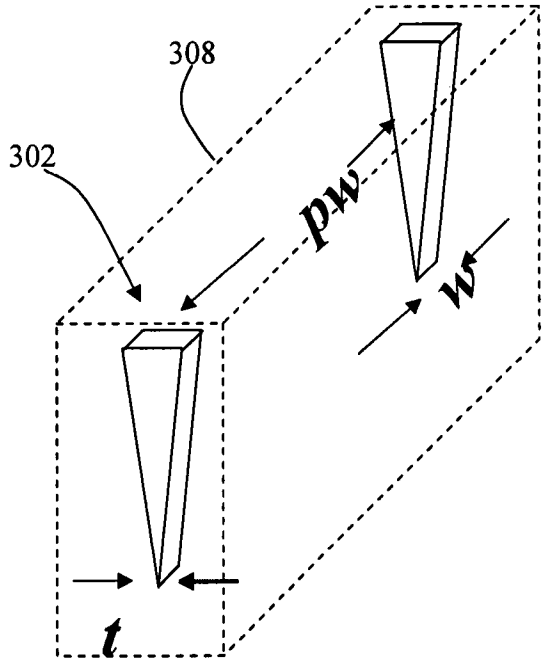


FIGURE 3

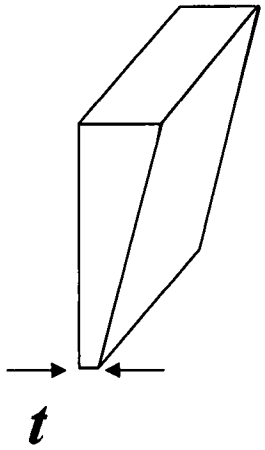


FIGURE 4A

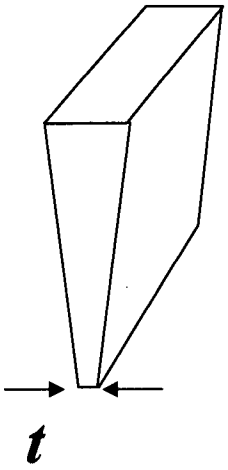


FIGURE 4B

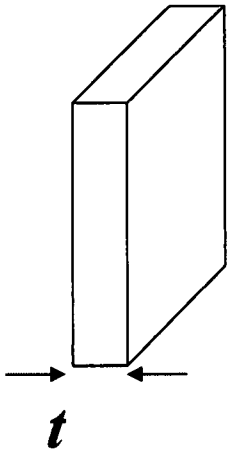


FIGURE 4C

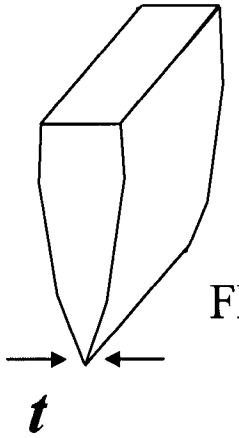


FIGURE 4D

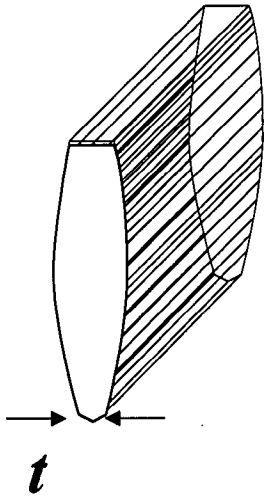


FIGURE 4E

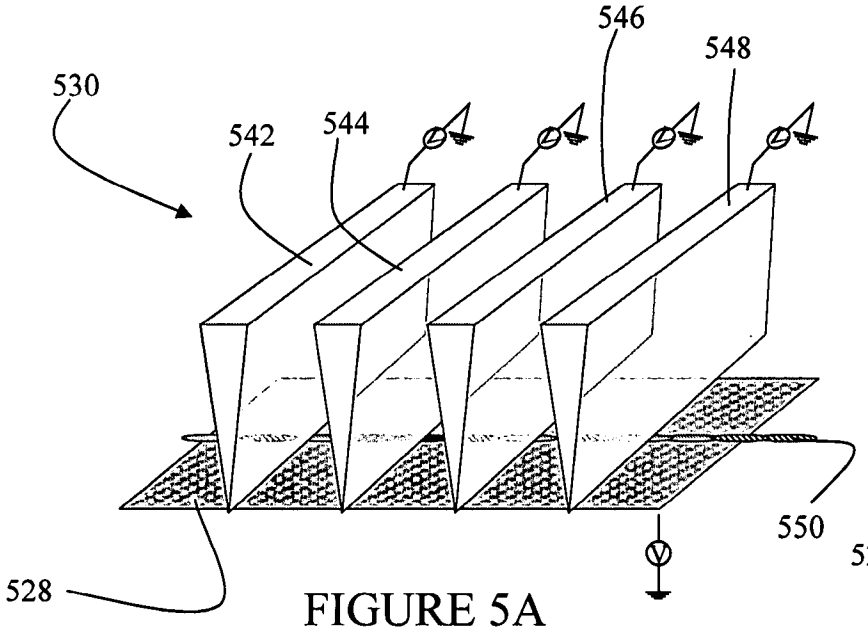


FIGURE 5A

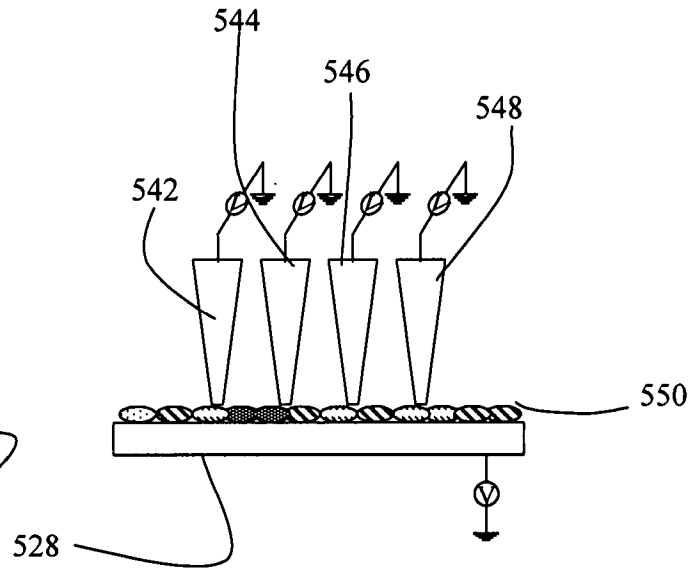


FIGURE 5B

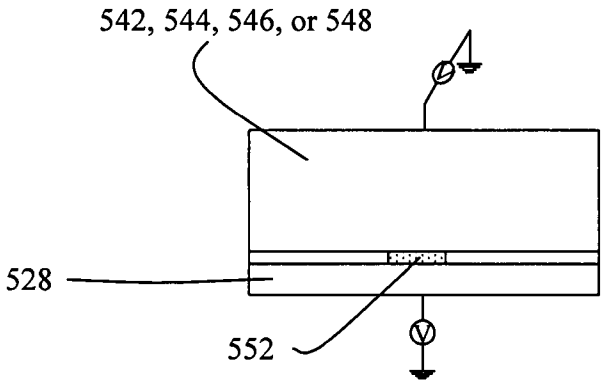


FIGURE 5C

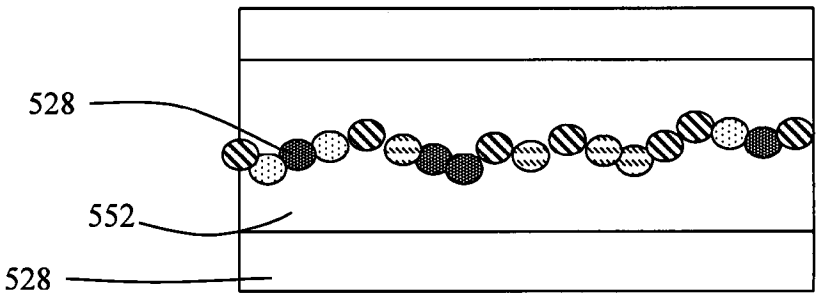


FIGURE 5D

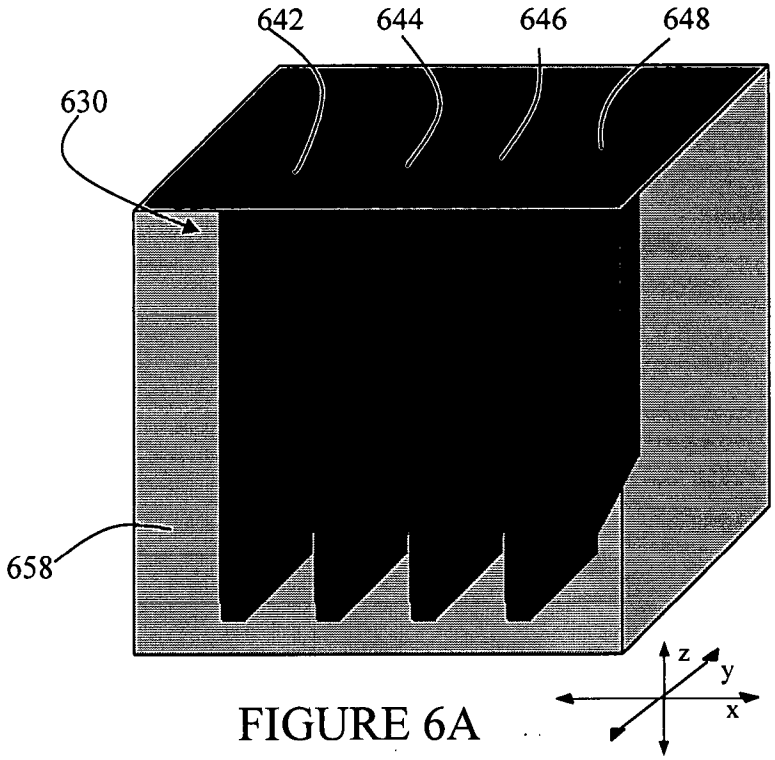


FIGURE 6A

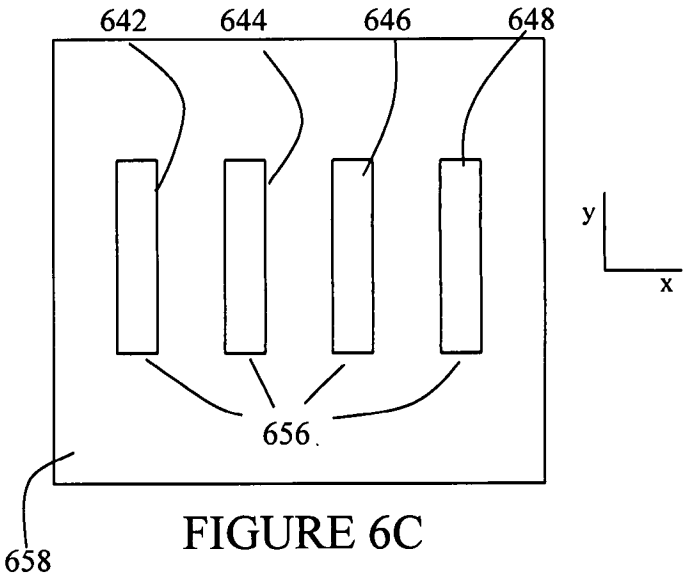


FIGURE 6C

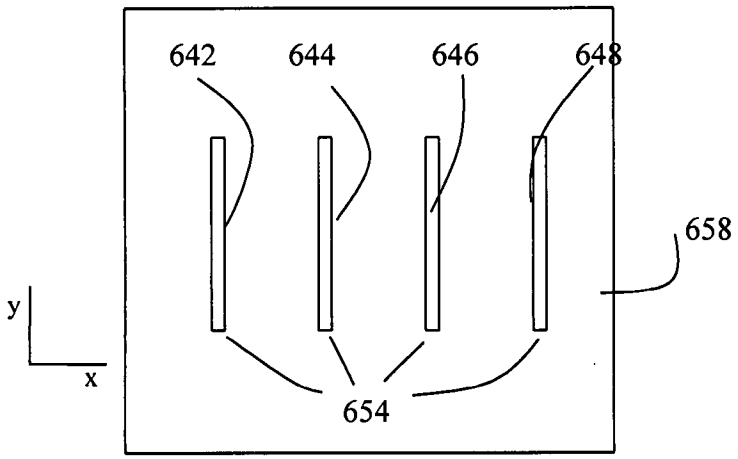


FIGURE 6B

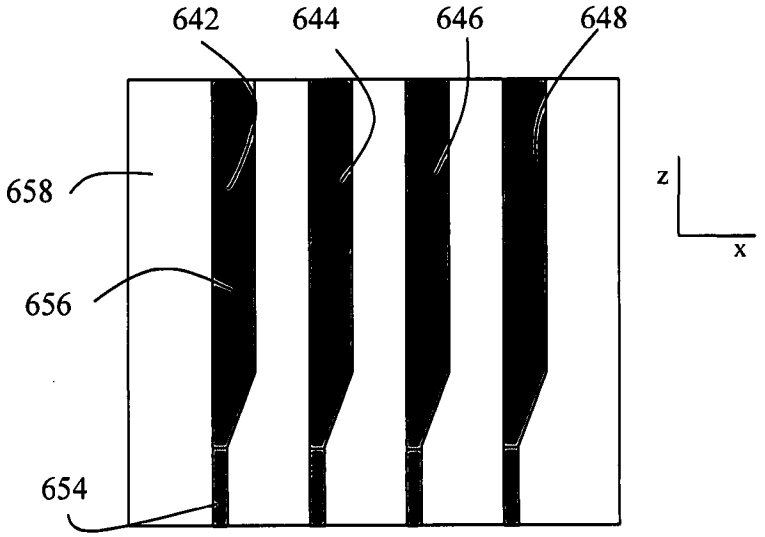


FIGURE 6D

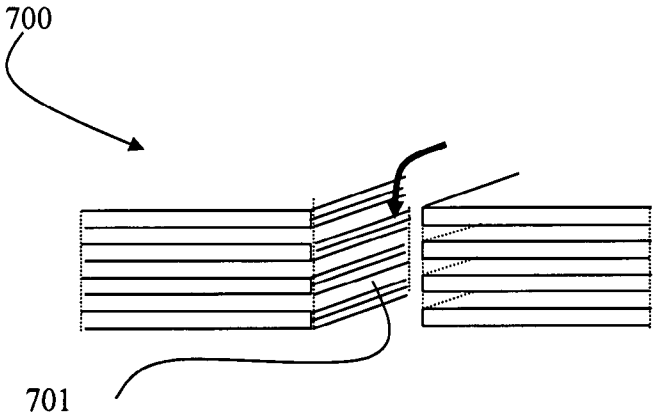


FIGURE 7A

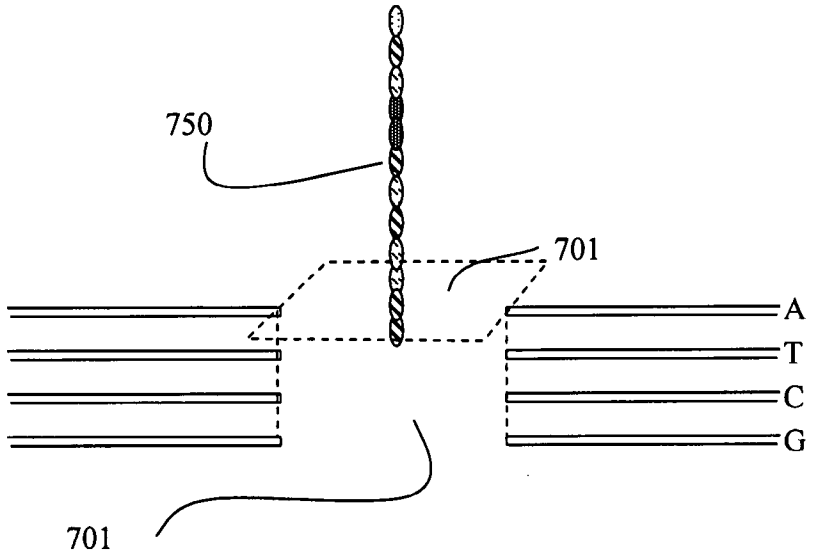


FIGURE 7B

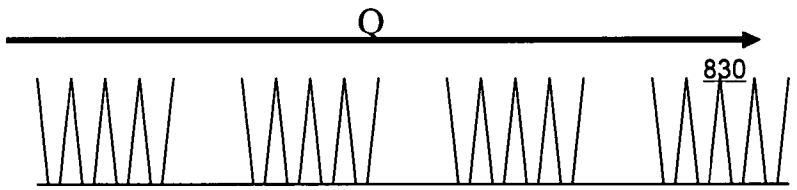


FIGURE 8A

877

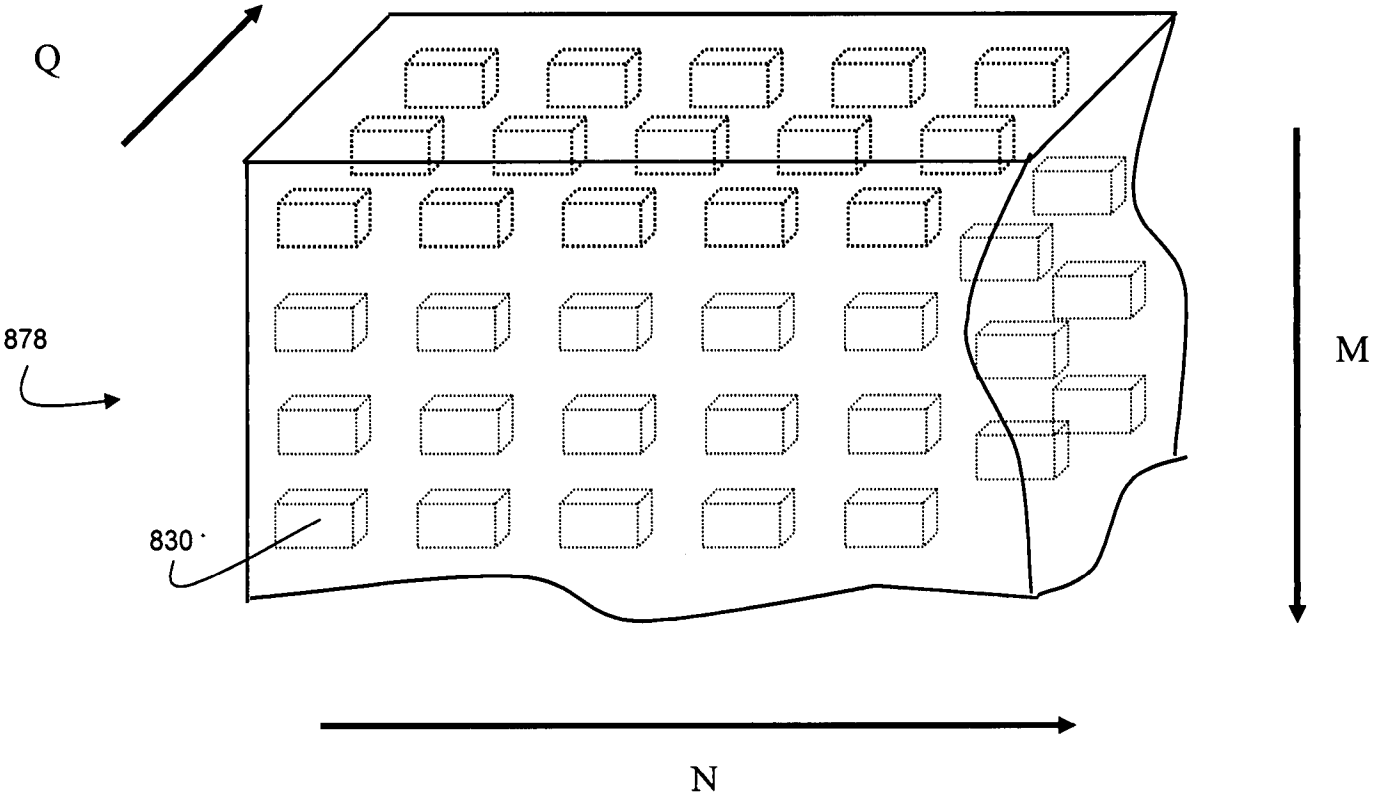


FIGURE 8B

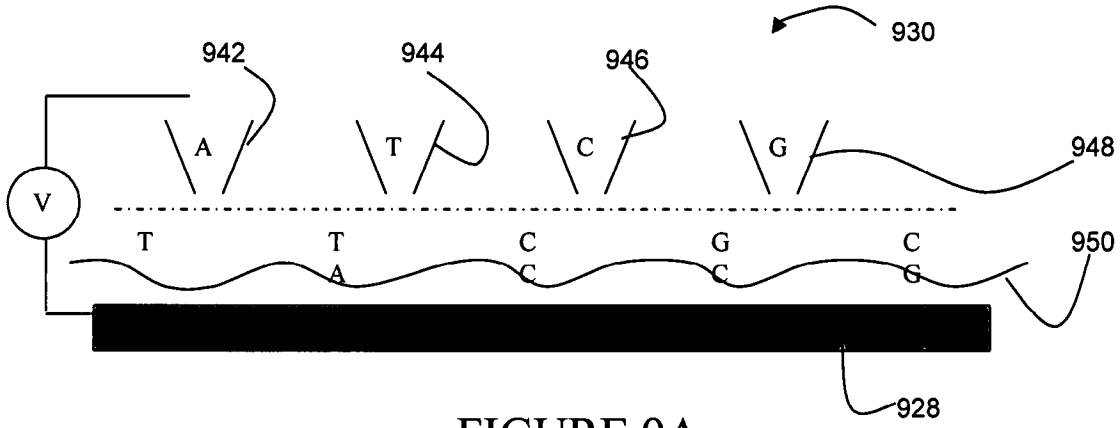


FIGURE 9A

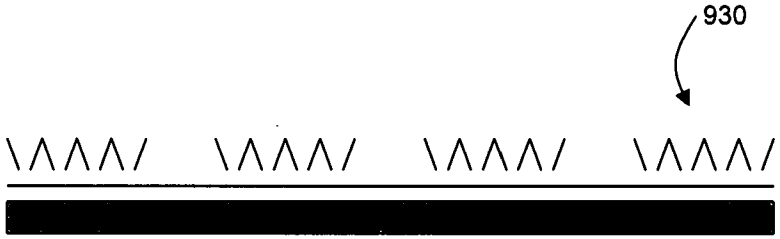


FIGURE 9B

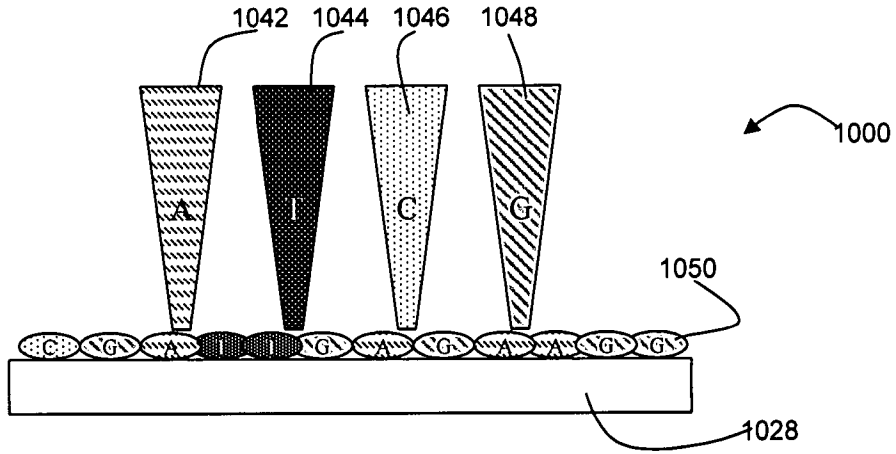


FIGURE 10A

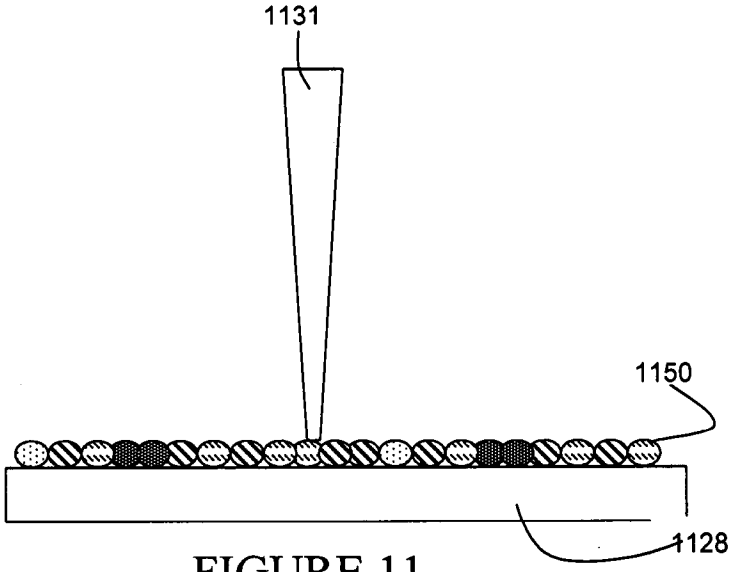


FIGURE 11

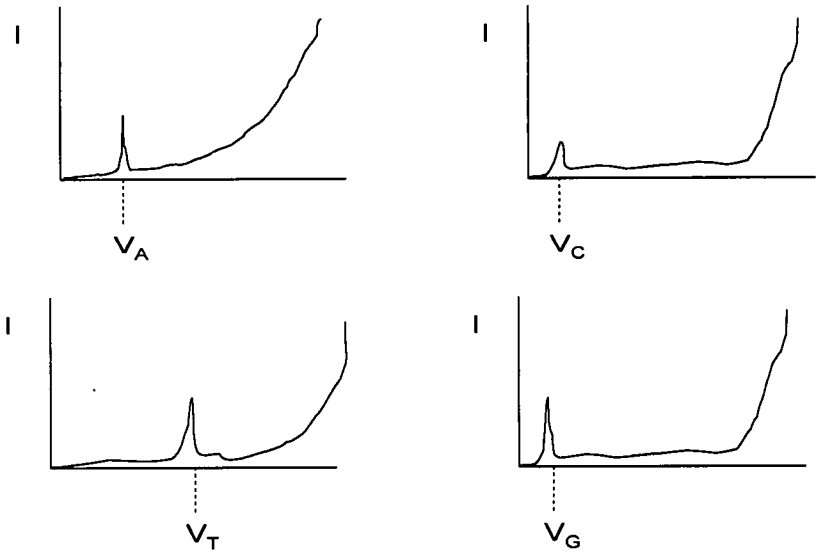


FIGURE 12

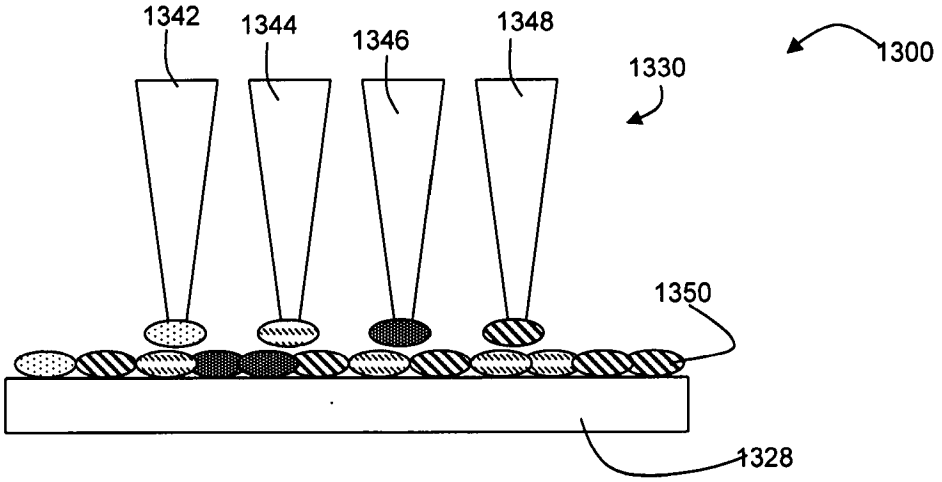


FIGURE 13A

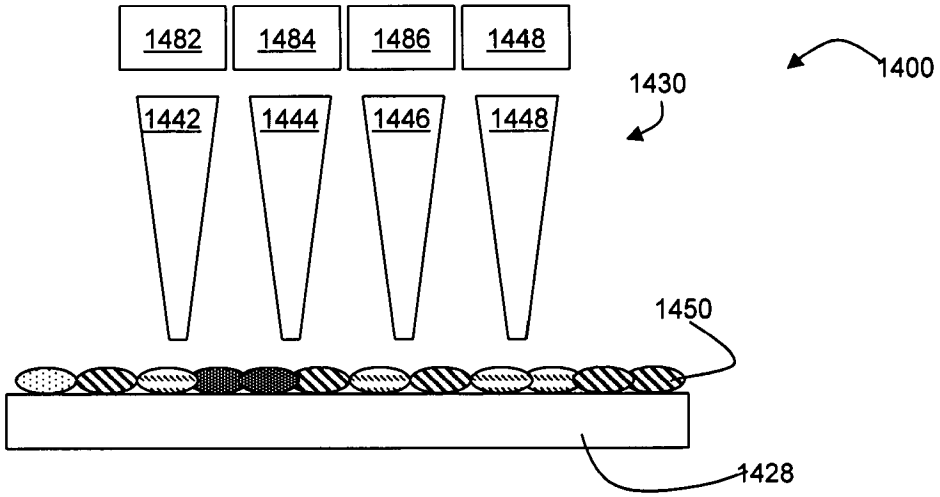


FIGURE 14

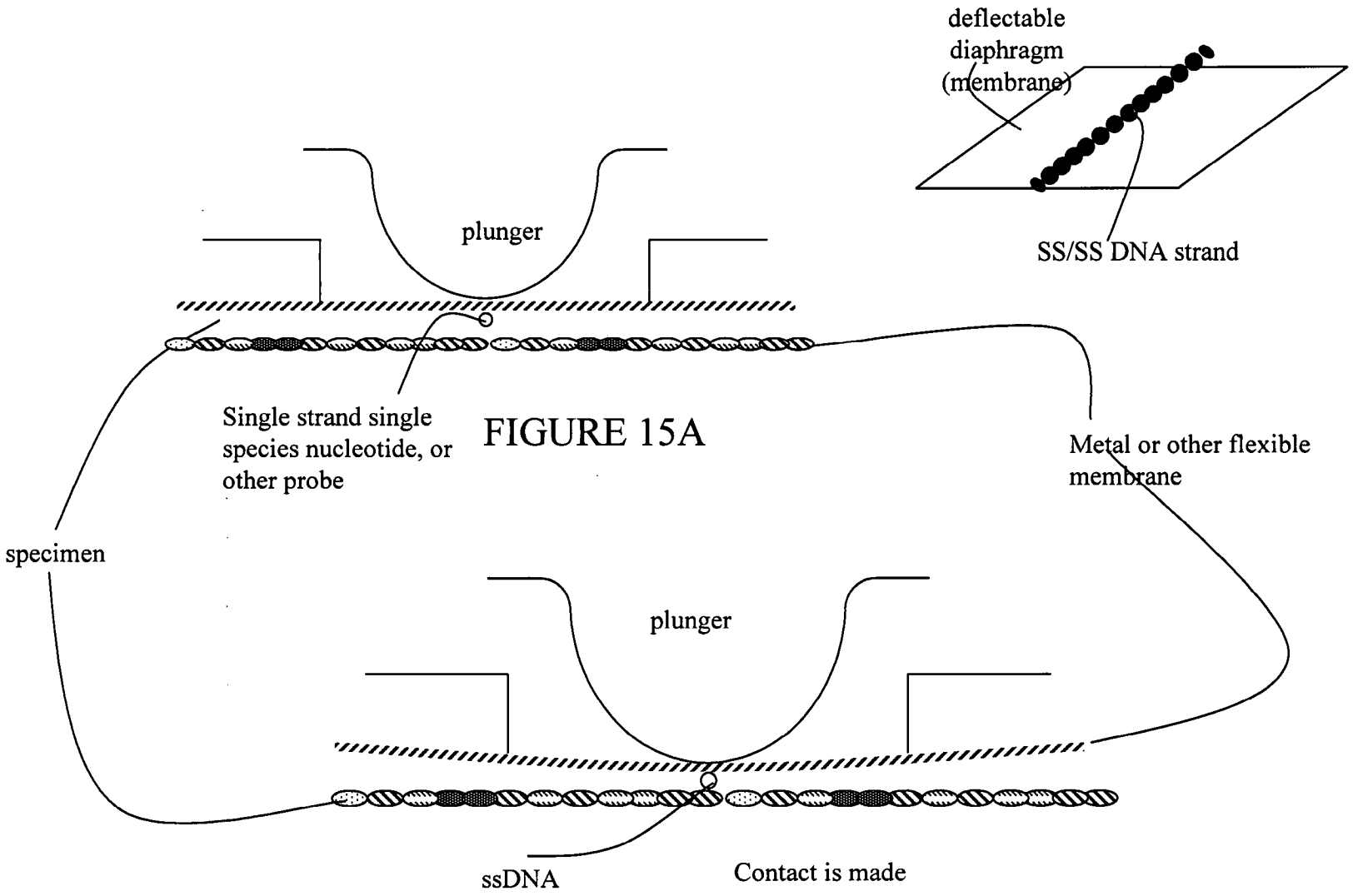


FIGURE 15A

FIGURE 15B

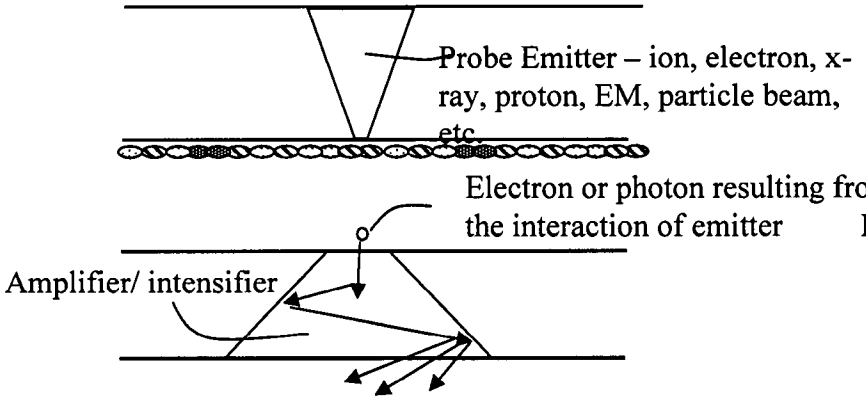


FIGURE 16A

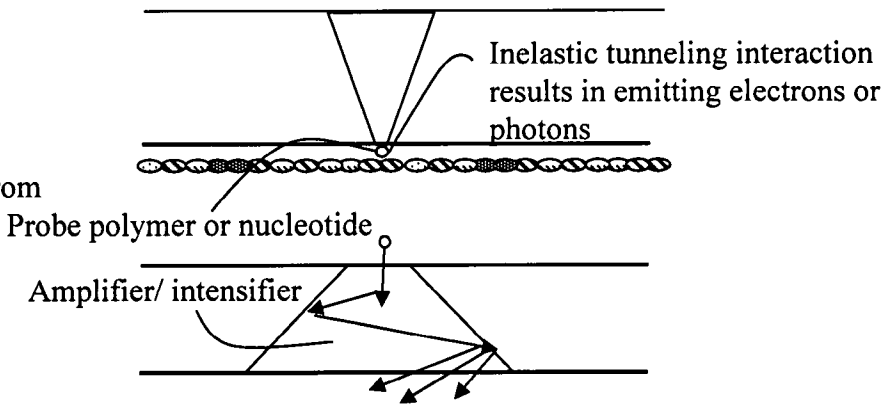


FIGURE 16B

Emission

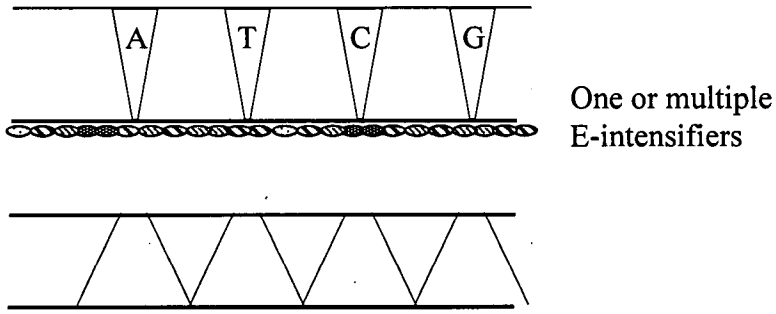


FIGURE 16C

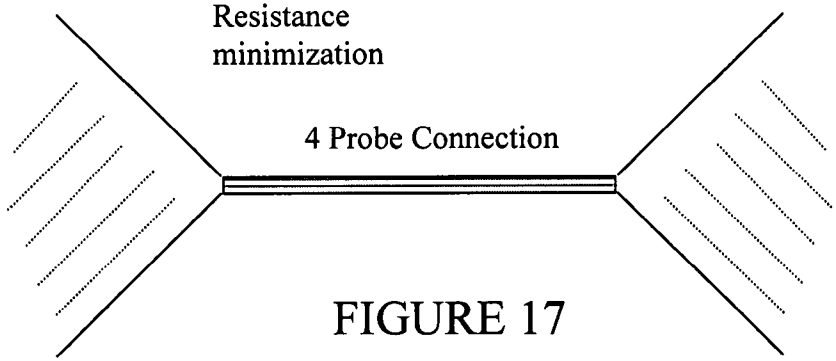


FIGURE 17

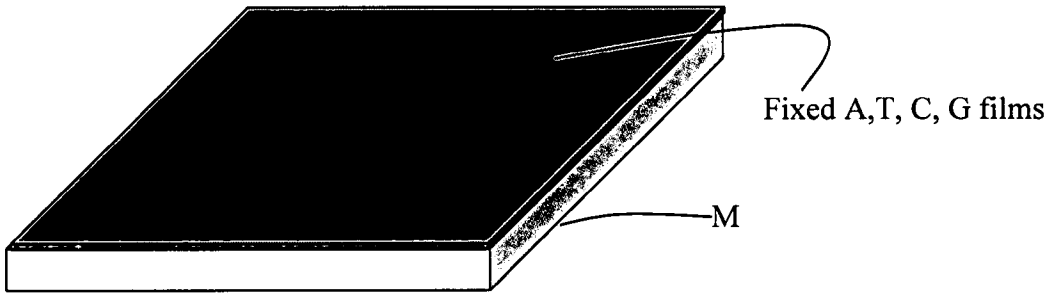


FIGURE 10B

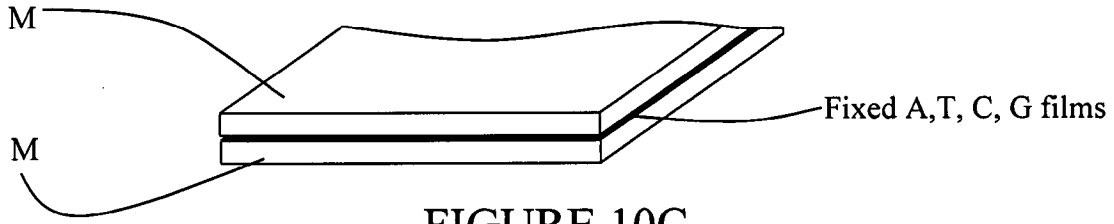


FIGURE 10C

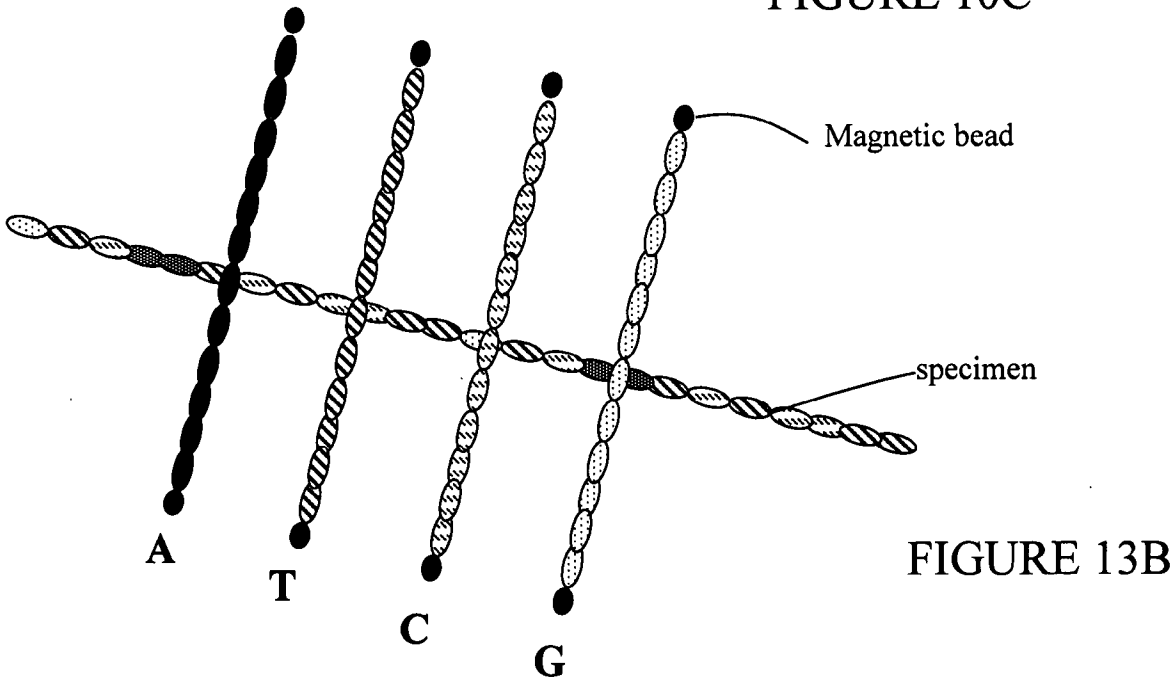


FIGURE 13B

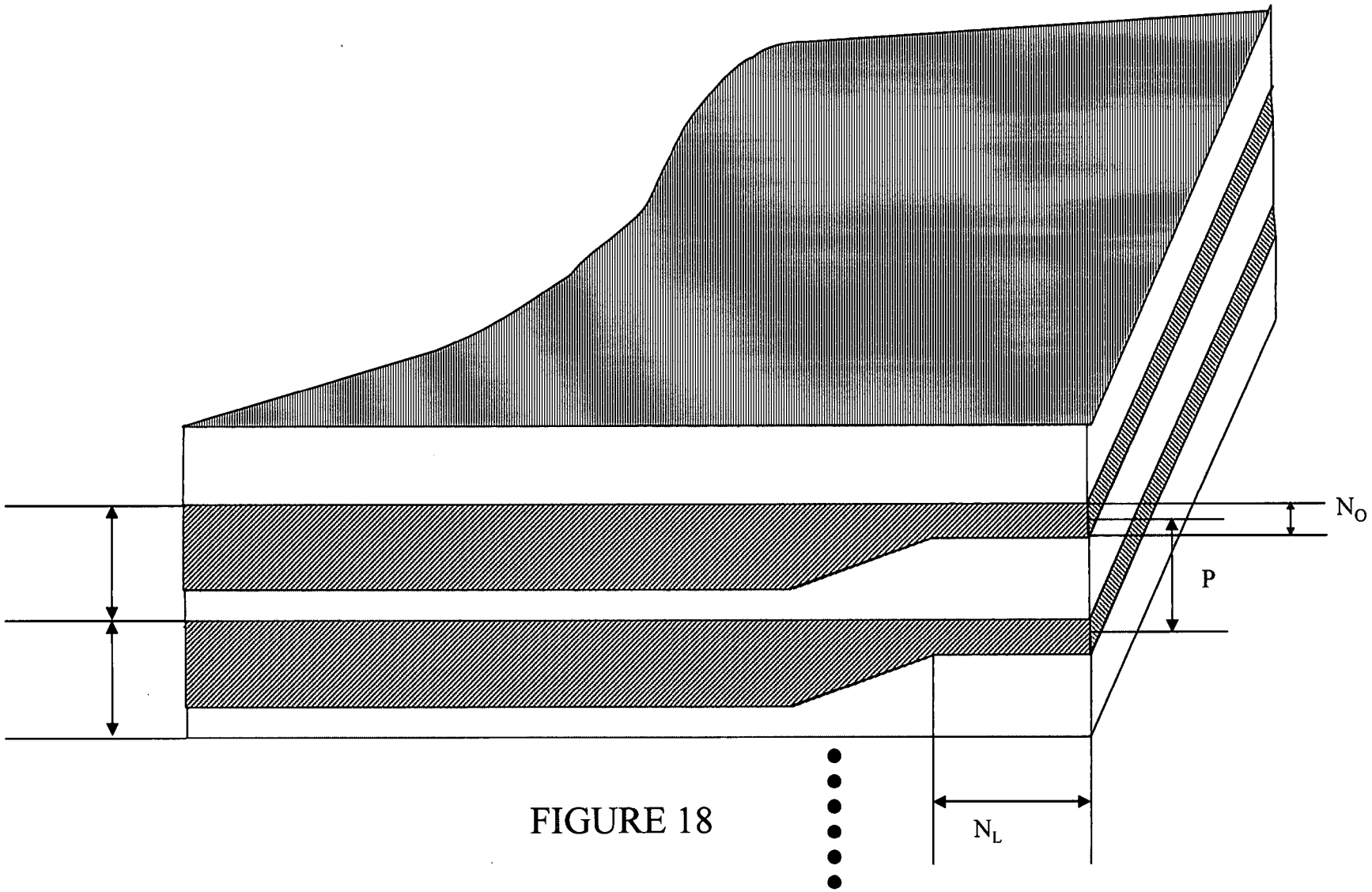


FIGURE 18

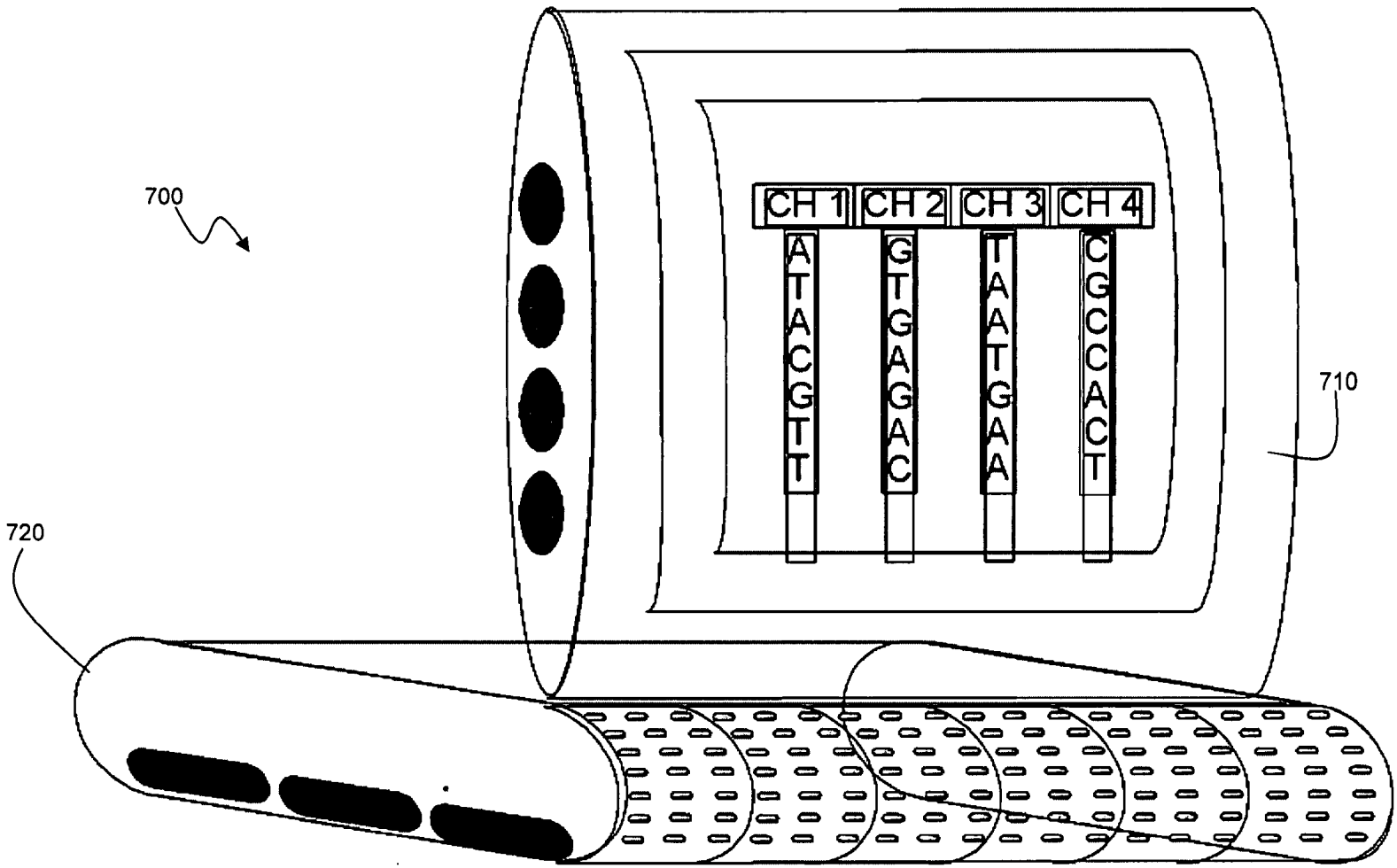


FIGURE 19

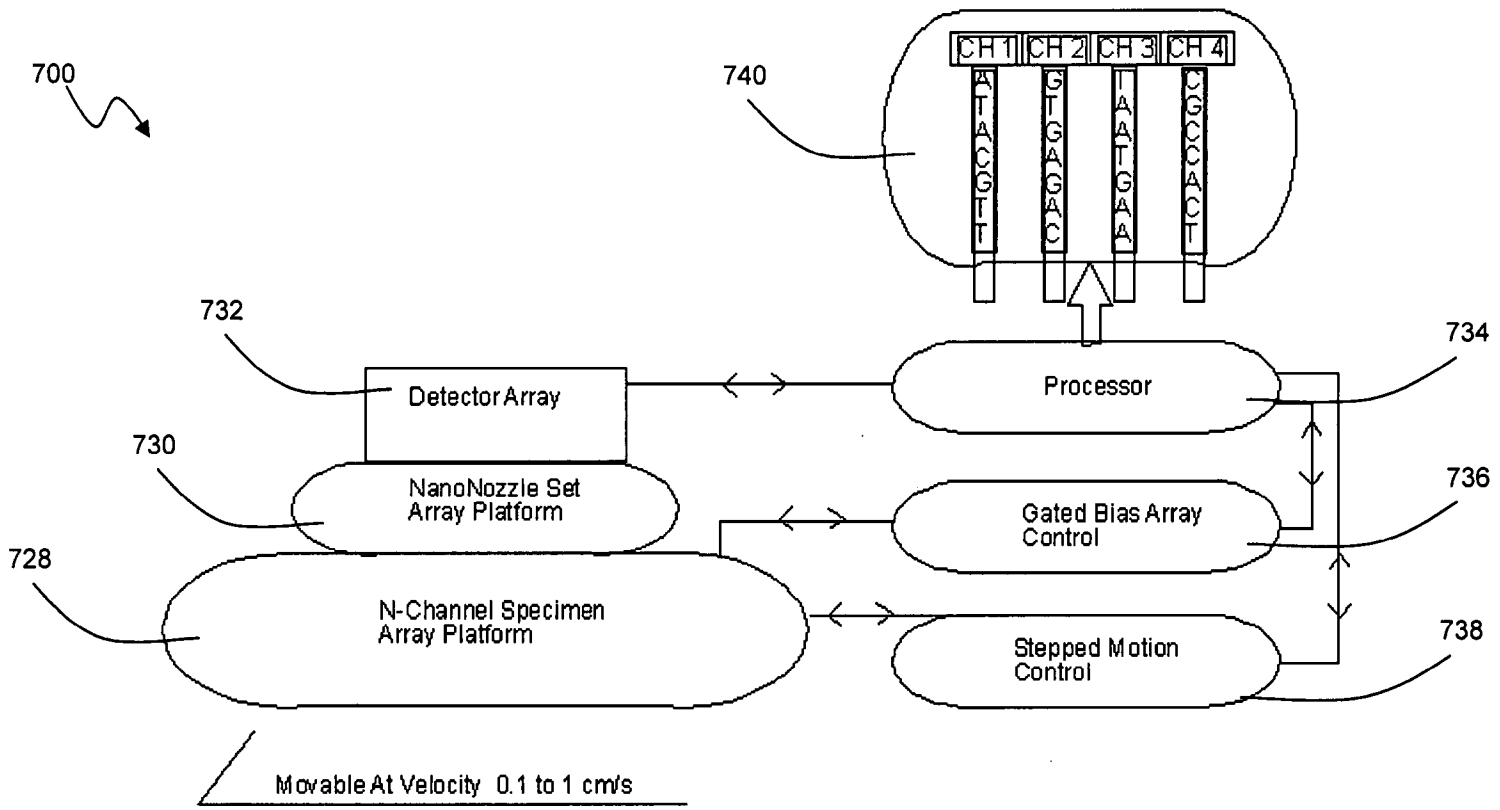

700
740
CH 1
CH 2
CH 3
CH 4
ATACGTT
GTGAGAC
TAATGAA
CGCCACT
732
Detector Array
734
Processor
730
NanoNozzle Set
Array Platform
736
Gated Bias Array
Control
728
N-Channel Specimen
Array Platform
738
Stepped Motion
Control
Movable At Velocity 0.1 to 1 cm/s

FIGURE 20

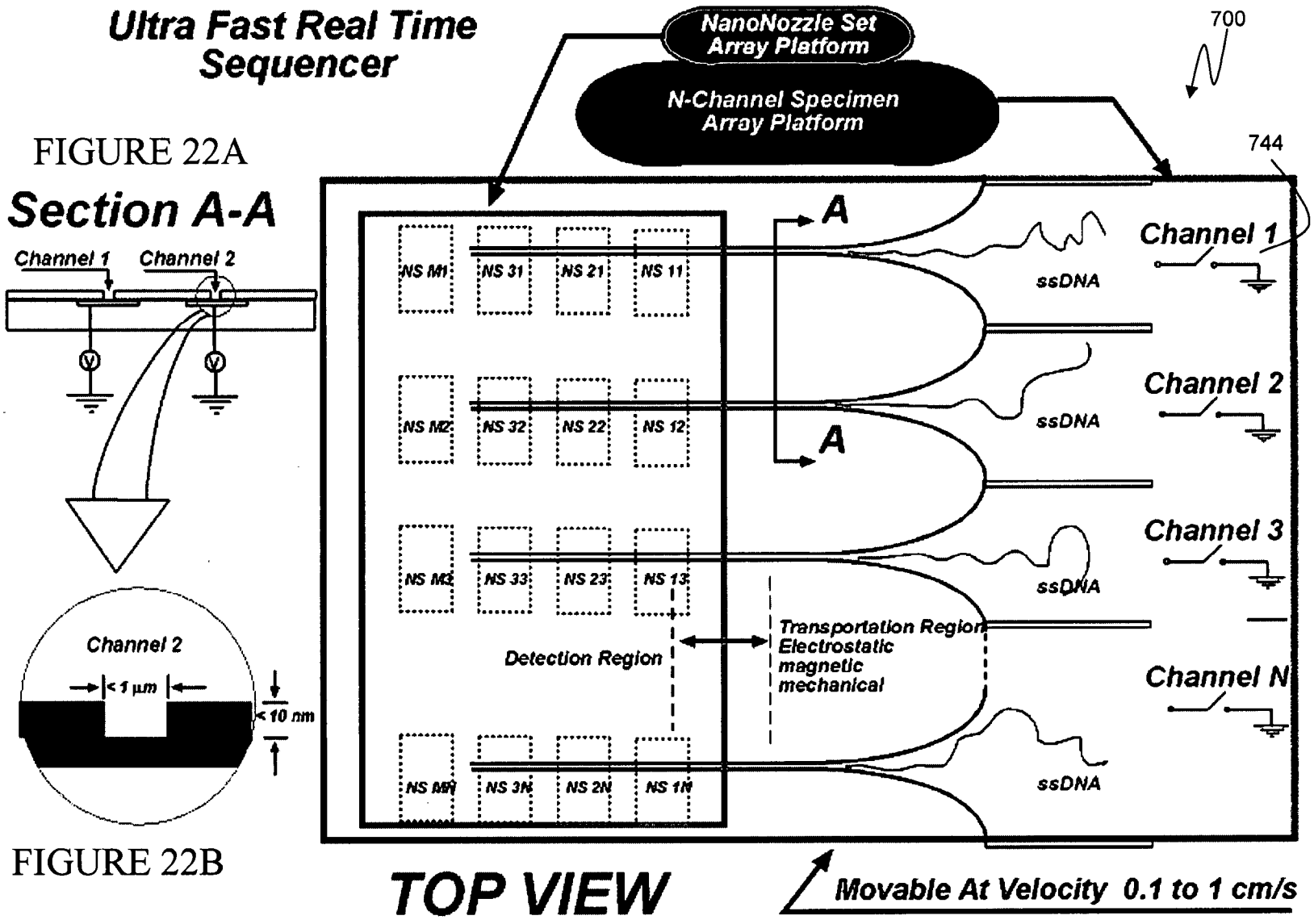


FIGURE 22A

FIGURE 22B

FIGURE 21

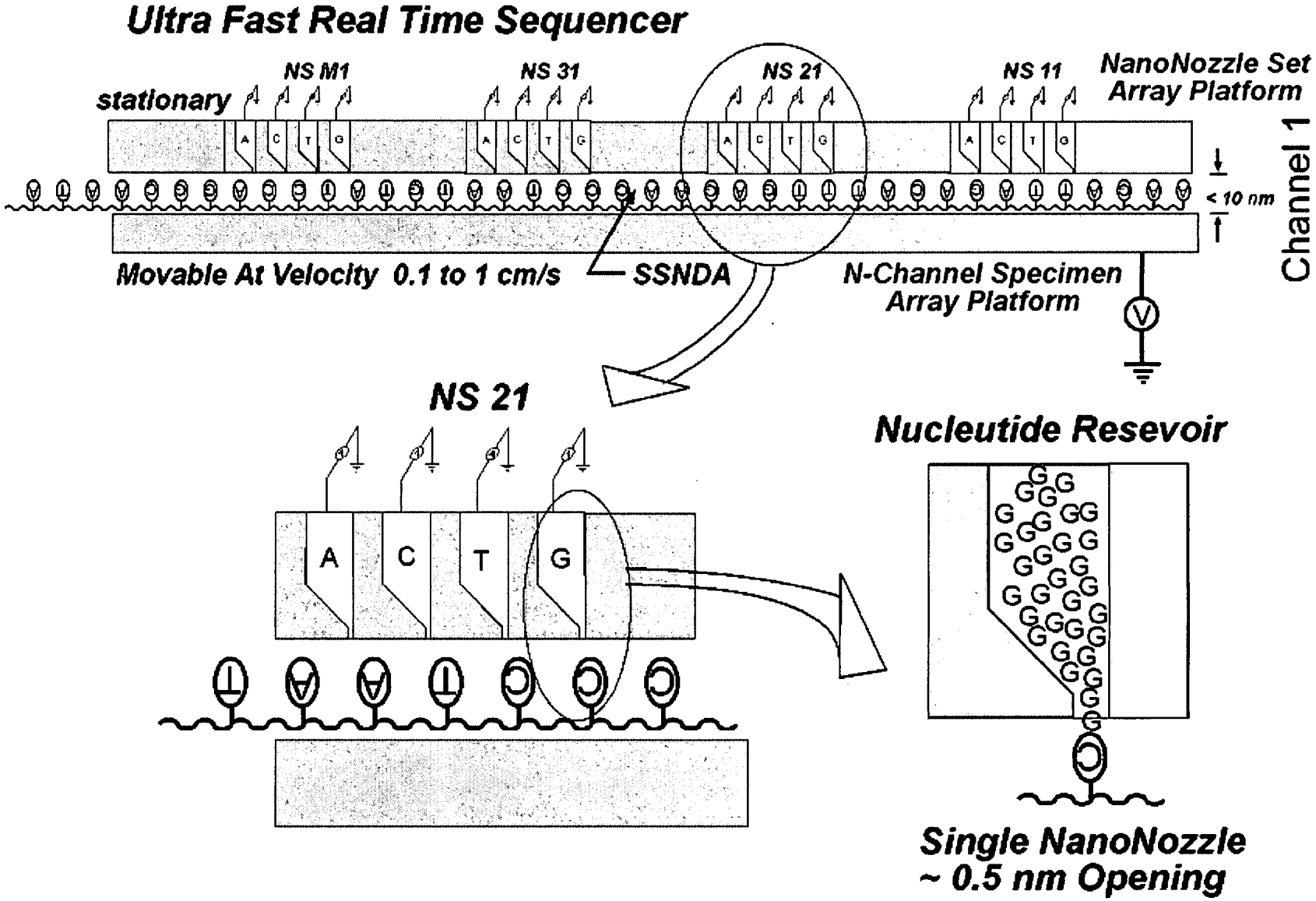

Ultra Fast Real Time Sequencer
NS M1
NS 31
NS 21
NS 11
NanoNozzle Set
Array Platform
stationary
A C T G
< 10 nm
Channel 1
Movable At Velocity 0.1 to 1 cm/s
SSNDA
N-Channel Specimen
Array Platform
NS 21
Nucleutide Resevoir
A C T G
Single NanoNozzle
~ 0.5 nm Opening

FIGURE 23

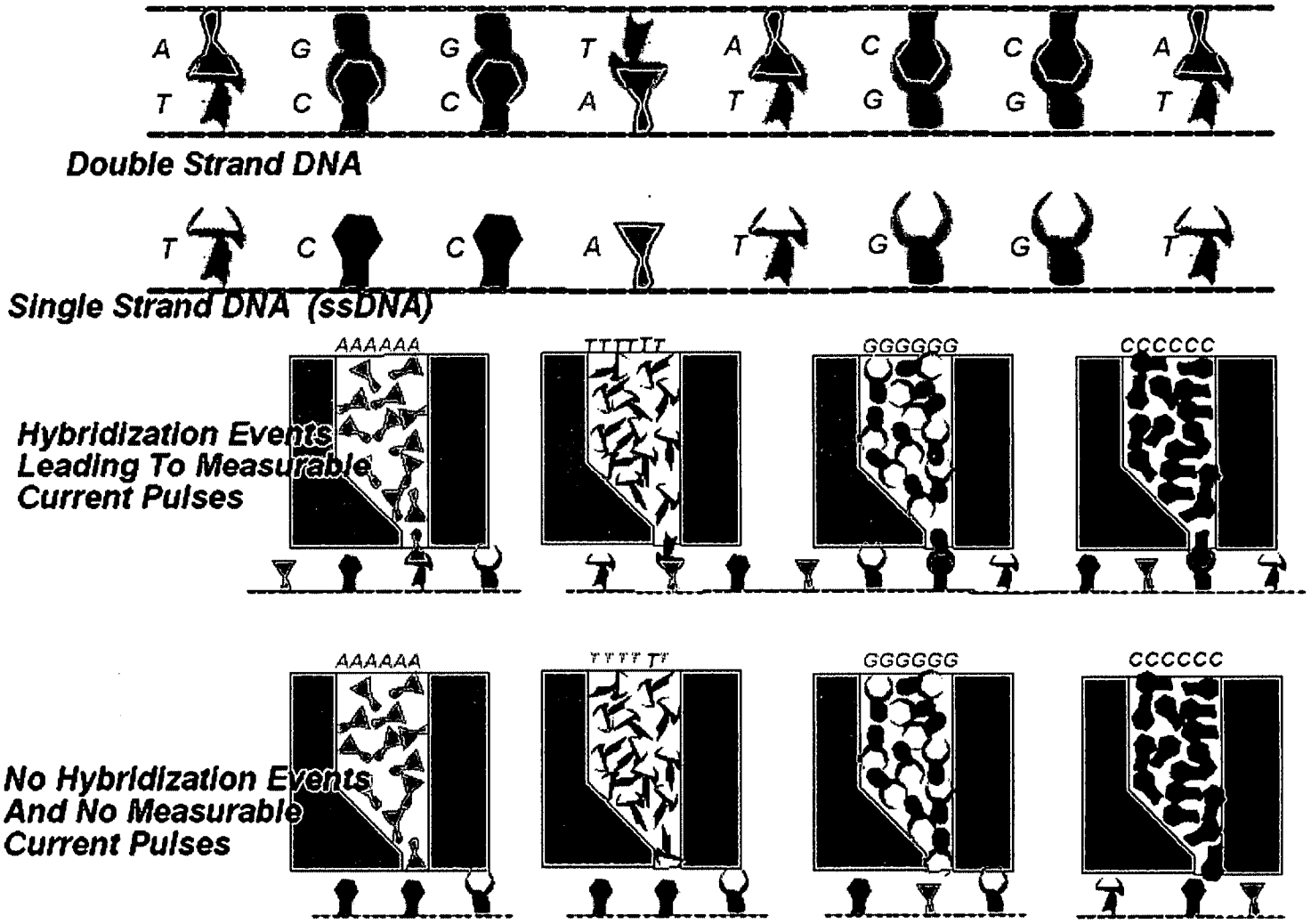

A G G T A C C A
T C C A T G G T
Double Strand DNA
T C C A T G G T
Single Strand DNA (ssDNA)
AAAAAA
TTTTTT
GGGGGG
CCCCCC
Hybridization Events Leading To Measurable Current Pulses
AAAAAA
GGGGGG
CCCCCC
No Hybridization Events And No Measurable Current Pulses

FIGURE 24

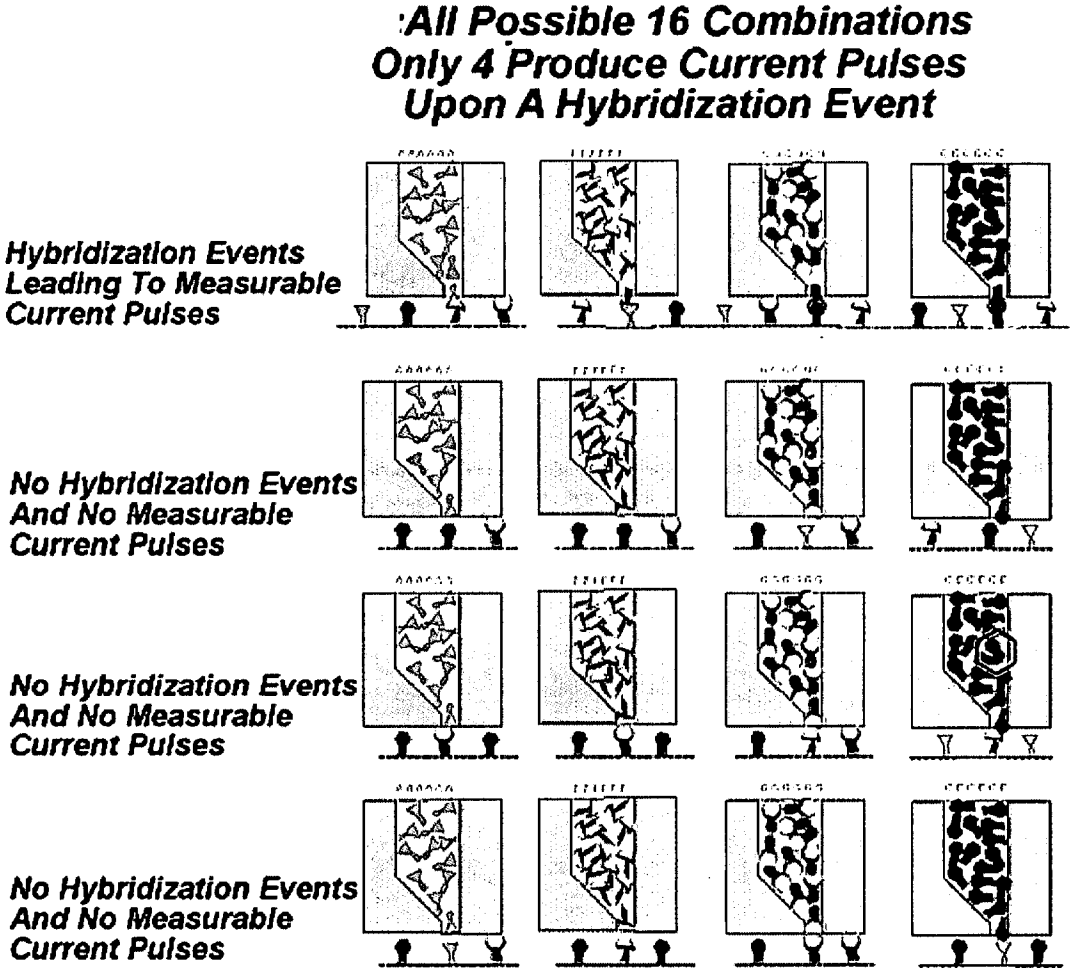
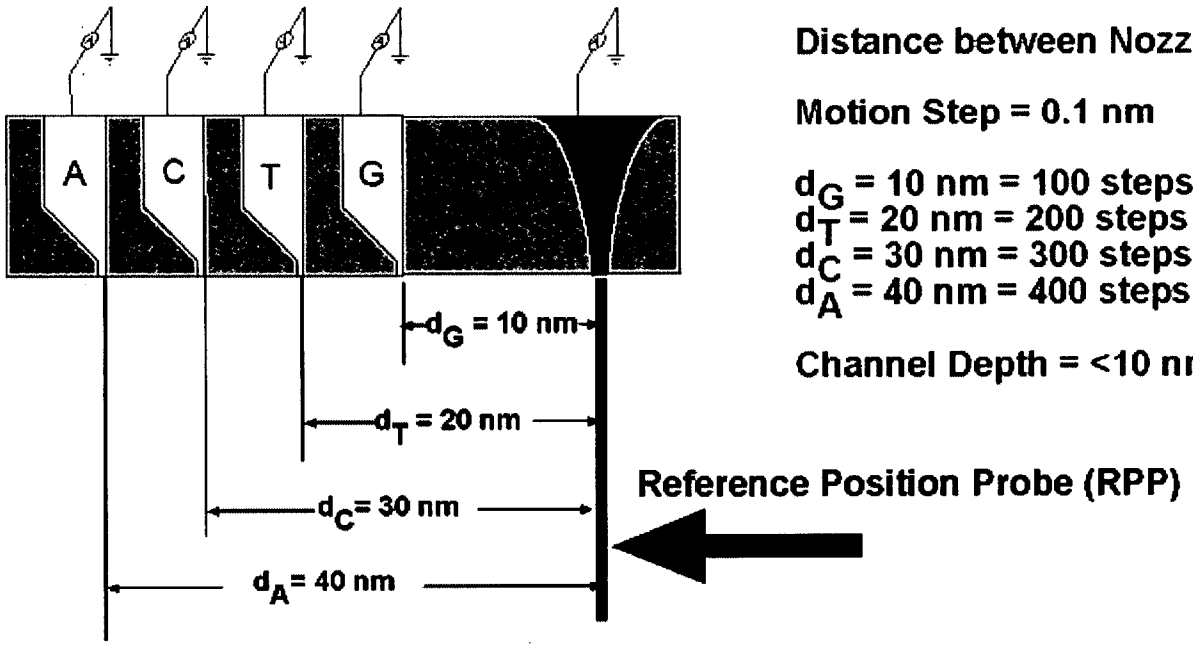

All Possible 16 Combinations
Only 4 Produce Current Pulses
Upon A Hybridization Event
Hybridization Events
Leading To Measurable
Current Pulses
No Hybridization Events
And No Measurable
Current Pulses
No Hybridization Events
And No Measurable
Current Pulses
No Hybridization Events
And No Measurable
Current Pulses

FIGURE 25

Reference Position And Precision nm Metrology



DNA base period p_b= 0.5 nm

Nozzle opening $x_N = p_b$= 0.5 nm

RPP size <0.5 nm

First Nozzle distance from RPP = 10 nm

Distance between Nozzles = 10 nm

Motion Step = 0.1 nm

d_G = 10 nm = 100 steps
d_T = 20 nm = 200 steps
d_C = 30 nm = 300 steps
d_A = 40 nm = 400 steps

Channel Depth = <10 nm

FIGURE 26

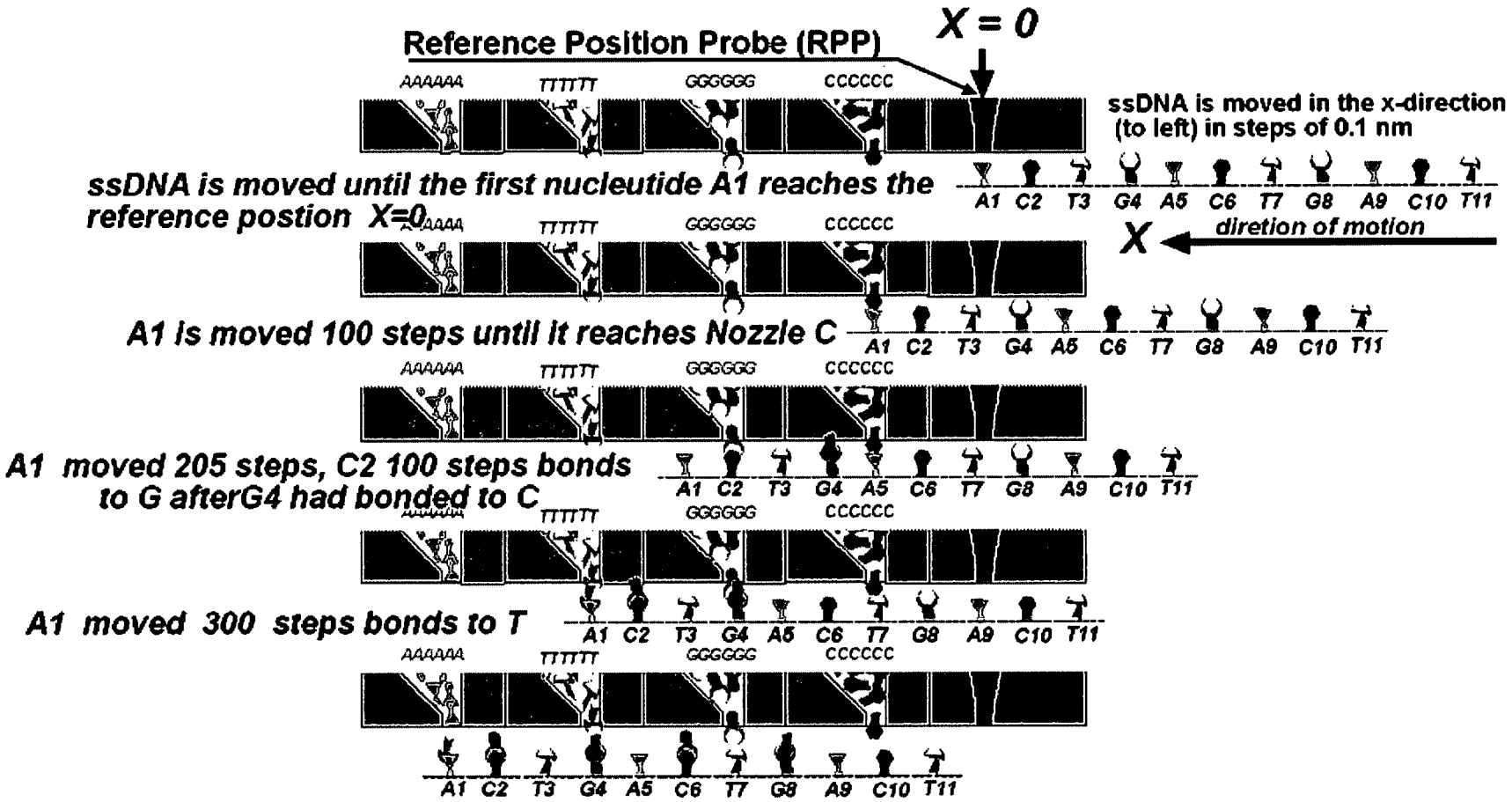

Reference Position Probe (RPP)
X = 0
AAAAAA
TTTTTT
GGGGGG
CCCCCC
ssDNA is moved in the x-direction (to left) in steps of 0.1 nm
ssDNA is moved until the first nucleutide A1 reaches the reference postion X=0
A1 C2 T3 G4 A5 C6 T7 G8 A9 C10 T11
X
diretion of motion
A1 is moved 100 steps until it reaches Nozzle C
A1 moved 205 steps, C2 100 steps bonds to G afterG4 had bonded to C
A1 moved 300 steps bonds to T
A1 moved 400 steps to Nozzle A. Note C6 and G8 had bonded

FIGURE 27